LOCAL (NONHORMONAL) FACTORS CONTROLLING BONE RECONSTRUCTION

Discussion Leader:

DR. ROBERT P. HEANEY

HEANEY: Although I am by nature an endocrinologist, I hope that we will mention hormones in this discussion only to exclude them from further consideration.

I would like to start with a quotation from the book, "Bone," by Dr. McLean and Dr. Urist (ref. 76); a brief paragraph entitled "Local Factors in Resorption" is as follows.

> The influence of local factors in the resorption of bone are most easily seen during its growth, when the metapnyses of the long bones are singled out for reconstruction. Such reconstruction invariably includes a considerable amount of osteoclastic resorption, followed by appositional bone formation, with the result that the size and shape of the bones progress toward the adult state. Organization and regulation of these phenomena, affecting most of the bones of the body simultaneously, is a part of overall growth. Some of the factors, at least, are local . . . .

It is these local factors to which we will direct our attention.

I think that although there is more remodeling going on in the skeleton than is characterized by the term "osteon," nonetheless this serves as a suitable temporary focus for some of our discussion. When remodeling occurs, as we all know, a space is tunneled out by osteoclasts, and it is only after this space has been created that new haversian bone is laid down within it. The localization of this space and its polarization in three dimensions are obviously not controlled by hormones.

FREMONT-SMITH: Is that haversian canal a very straight line?

HEANEY: It tends to spiral.

FREMONT-SMITH: To spiral?

HEANEY: A very gradual spiral, mostly longitudinal.

FREMONT-SMITH: Of about what length? A few millimeters, or is it longer?

MCLEAN: Cohen and Harris (ref. 77) did a series of reconstructions—wax models—from serial sections through bone, and they outlined the dimensions and the form of these canals quite accurately. This is the one standard we can look at. A single osteon may be as much as 2 millimeters in length.

FREMONT-SMITH: Thank you.

HEANEY: Although parathyroid hormone, cortisol, growth hormone, thyroid hormone, and a variety of others may well have some effect on the activity of the cells carrying out these processes, obviously the hormone levels are inadequate to explain both the localization and the polarization of these cellular activities, so let us exclude hormones from formal consideration.

BAUER: When you say "polarization," do you mean spatial distribution.

HEANEY: No; I mean more than spatial distribution. When a cutting cone of osteoclasts begins to tunnel out a haversian cavity, the cone proceeds in a definite direction.

BAUER: Distributed in space, anyway; that is what you mean.

HEANEY: Osteoblasts are recruited from their progenitor cells to lay down bone at very specific places within the bone, at specific rates, and in specific quantities. Similarly osteoclasts are recruited; they resorb bone, and they do so at specific places and at specific rates. That they do this is an activity proper to bone simply because it is bone.

To be very certain that everybody knows exactly what I hope we shall talk about in this discussion, I would like to give an example from a completely different field, the rhythmic contractility of a tissue such as cardiac muscle.

We know that skeletal muscle in the resting stage is able to maintain a gradient of sodium and potassium across its cell membrane virtually indefinitely, whereas cardiac muscle has an intrinsically leaky membrane. The sodium ion tends to leak across the cell membrane until a critical gradient is reached; at this point depolarization is triggered, the cell is activated, and then during the recovery phase the sodium is pumped back out. This occurs spontaneously, and the period of this rhythm is determined, obviously, by the leakiness of the cell membrane.

The rate at which the heart works is determined by nerves, hormones, humoral agents, drugs, and one thing or another which act on this system; but the important point is the fact that all of these agents act upon this intrinsic rhythmic contractile system. Rather than directing our attention to the regulatory factors of a higher order of magnitude, such as the nerves and the hormones themselves that circulate through the blood and influence cardiac rate, let us direct our attention to the mechanism on which these factors play; that is, the intrinsic, rhythmic nature of the contractile system itself.

In bone we are not concerned with a rhythmic cell depolarization—at least I do not know that we are—but we are concerned with an equally intrinsic sort of tissue activity—the reconstruction of bone tissue as tissue. We have discussed the effects of a variety of recognized hormones on bone in the homeostasis of the extracellular fluid

calcium. Now, I would like to descend one whole order of magnitude to the tissue mechanism, not the biochemical mechanisms per se, but the tissue mechanisms on which these various hormonal factors play. I think this approach has a practical importance aside from the theoretic and biologic significance because these local factors exert very prominent effects on metabolic bone disease; perhaps we have not always looked far enough and wide enough to recognize their existence.

I first began to think seriously about this matter 10 years ago when working with Dr. Whedon at the National Institutes of Health. We came upon some unexpected results in an experiment. (See ref. 78.)

I will try to avoid going into a lot of detail about this experiment because the nature of the experimental system is not germane to the point which I want to make. Suffice it to say that we had calcium and nitrogen balances on a patient with active rheumatoid arthritis prior to and during treatment with prednisolone (fig. 42).

WHEDON: The patient also had osteoporosis.

HEANEY: Yes; that is in the record.

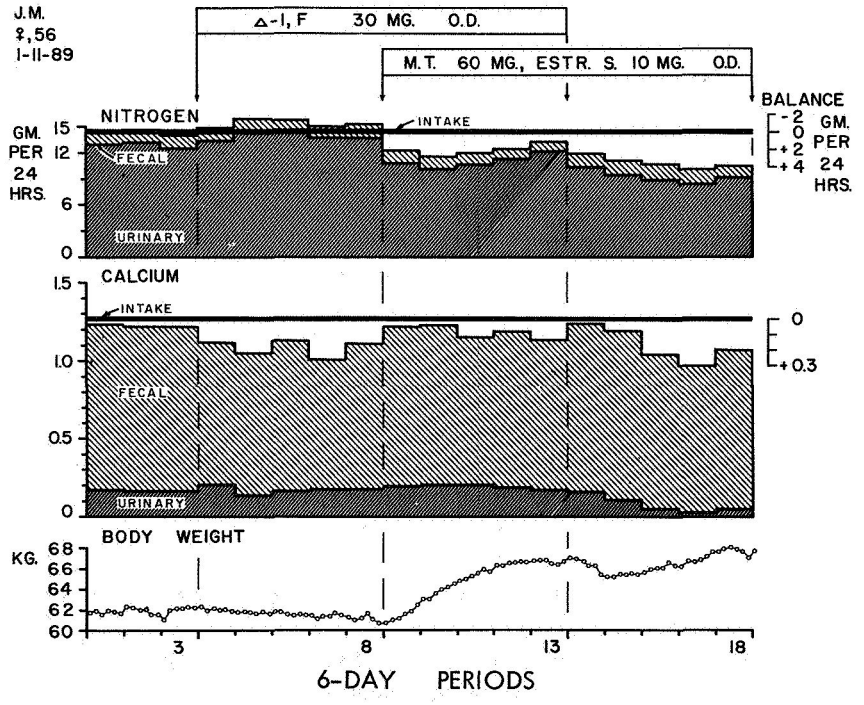

FIGURE 42. Nitrogen and calcium balances and changes in body weight in a 56-year-old woman with active rheumatoid arthritis and osteoporosis of the spine. [From ref. 78; reprinted by permission of the author.]

WHEDON: I mentioned that because it has everything to do with how it all comes out or does not come out, according to how you expect it should.

HEANEY: Again, I would rather not explain or attempt to explain the very complicated system here. Suffice it to say that during the control period this patient was in approximate nitrogen equilibrium with a very slight positive calcium balance on a high calcium intake. We had anticipated, perhaps naively, that when we put this patient on a high dose of corticosteroids, she would go into negative calcium balance. This is expected in normal people. It is certainly a feature in spontaneously occurring Cushing's disease and so forth. But instead of going into negative calcium balance, the patient had, in fact, increased her calcium storage during the period of medication.

This seemed paradoxical to us at the time and still does to me, although I have a number of explanations for it which I will avoid talking about at this time because they are not germane to the point of the discussion.

This observation has been repeated many times. It is not a uniform observation and cannot be found in all patients with rheumatoid arthritis or in rheumatoid arthritis with osteoporosis; but it does occur in many arthritic patients, and it has been reported by other people as well. It suggested to us that there may be some difference in the bone that is adjacent to active rheumatoid arthritic inflammation.

Calcium kinetic studies performed in patients of this sort have demonstrated the expected cortisol or corticosteroid effect; that is, a decrease in total body structural turnover of bone. This effect is difficult to explain in view of this positive balance shift. Certainly, clinically one expects the osteoporosis, if nothing else, to not get better and perhaps to become worse.

These observations prompted a number of other experiments. To make a long story short, we have been able to make rough measurements of bone accretion rates, as defined by Bauer et al. (ref. 79), in isolated segments of the intact limbs of patients with rheumatoid arthritis (fig. 43). The numbers along the abscissa represent successive 3-inch segments from the tip of the fingers on up the arm. They are numbered consecutively for convenience. The metacarpal-phalangeal region would be No. 2 and the wrist region, No. 3. The control is shown in solid black and prednisolone treatment, cross-hatched.

Even though in this patient, as a whole, the total body accretion rate fell as a result of corticosteroid treatment, there was no decrease in the bone mineral accretion rate in these segments during corticosteroid treatment. This experiment has been repeated many times and is a uniform finding.

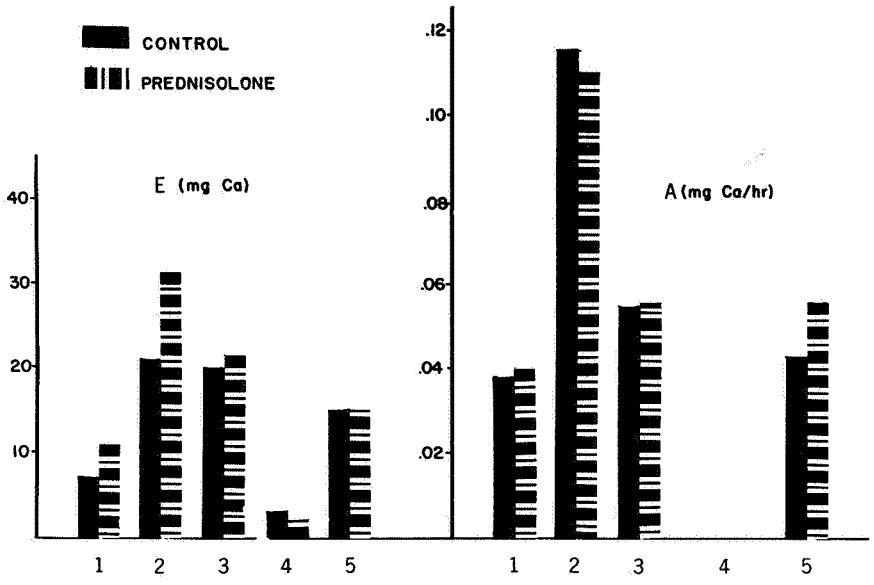

FIGURE 43. Bone accretion rates in patients with rheumatoid arthritis.

It is not apparent in this isolated figure, but the levels of accretion shown are from two to five times what one would find in normal people; thus there is increased structural turnover of bone around these inflamed joints, and this increased structural turnover is not depressed, at least over our periods of observation which are in weeks or months, by corticosteroid treatment, even though the sum total of all the bone in the patient's body did show a net decrease in bone mineral accretion. This emphasizes, I think, that there was a local factor in the bone adjacent to the inflammation, a local factor which was more important than, or which modified, the expected bone tissue response to a superimposed systemic or hormonal controlling agent.

URIST: Do these data mean that the positive accretion occurred in the area of the involved joints?

HEANEY: By positive accretion you mean positive calcium balance, increased bone mass?

URIST: Yes.

HEANEY: I did not measure this. It would be exceedingly difficult to measure, short of very sensitive densitometric methods which I have not employed. What I have measured is the bone accretion rate by these techniques, in localized segments.

URIST: Name the area included in each segment.

HEANEY: Around and including the involved arthritic joints, but the accretion is, I assume, going on in the bone rather than in the joints.

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HOWELL: How old were these patients that you studied? In older rheumatoid arthritic patients, there may be considerable hypertrophic bony growth at the margins of the joints; this growth might affect mineral accretion in one direction. In adjacent subarticular bone, osteoporosis is a common early finding, and I should think that it is there that one might expect to register quite different mineral accretion rates.

HEANEY: These patients had osteoporosis, as observed by X-ray, but it does not matter for my purpose in this discussion that they did, because whatever it was they were doing, it was not depressed by an effective therapeutic dose of corticosteroid. This is the only point that I want to make. These local regions did not respond as the skeleton at large did in the same patient, nor did they respond in the way one has become accustomed to expect normal bone to respond.

NICHOLS: Do we know how normal bone responds to cortisone in a biochemical sense at the cellular level?

HEANEY: No. I am not talking about cellular level. I want to steer between the hormonal and the biochemical level and, somehow, end up at the tissue level itself.

LLOYD: Could you give us some more information as to how you calculated the "A" values for these particular cases?

HEANEY: I would like to but will resist the temptation because it would be a digression. I would be glad to do so at a later date when we have more time.

I am attempting to give examples of what I consider to be the effects of local factors. After I have given a few of these examples, I shall ask Dr. Talmage to give some further examples. We were interested in some of the physiologic mechanisms involved in the development of disuse osteoporosis. In one experiment designed to attack this problem, we cut all of the nerves in the lumbar plexus on one side of 200-gram young-adult rats; this caused marked muscle atrophy of one leg together with disuse osteoporosis on that side, rapidly developing over a period from 4 days to 3 weeks after nerve section. During this time we took measurements of the calcium content of the bones concerned and measurements of the uptake of radioactive calcium given at varying periods prior to sacrifice.

I will not go into any further detail about this aspect of the experiment because I do not want to talk about disuse osteoporosis. I do want to talk about some local factors and how they influenced our results—not to explain these factors but to show how they influenced our results.

We separated the bones into four regions. First, we took the femoral head and intertrochanteric region. We had to cut this off somewhat arbitrarily, but tried to be as precise as we could. Second,

we studied the remainder of the femoral shaft. Then we studied the knee, which consisted of the distal epiphysis of the femur and the proximal epiphysis of the tibia. These were readily separable because one could simply snap them off at the existing cartilage plates. These were almost fully grown rats so that the plates were not very active, but we could nevertheless separate them. Finally, we studied the remainder of the tibia-fibula complex.

Figure 44 is schematic and illustrates the changes in total calcium and in ^{45}Ca uptake in these various regions. The top line deals with denervation alone. I will touch on the others in a moment, although they are not as important as the point I want to make here.

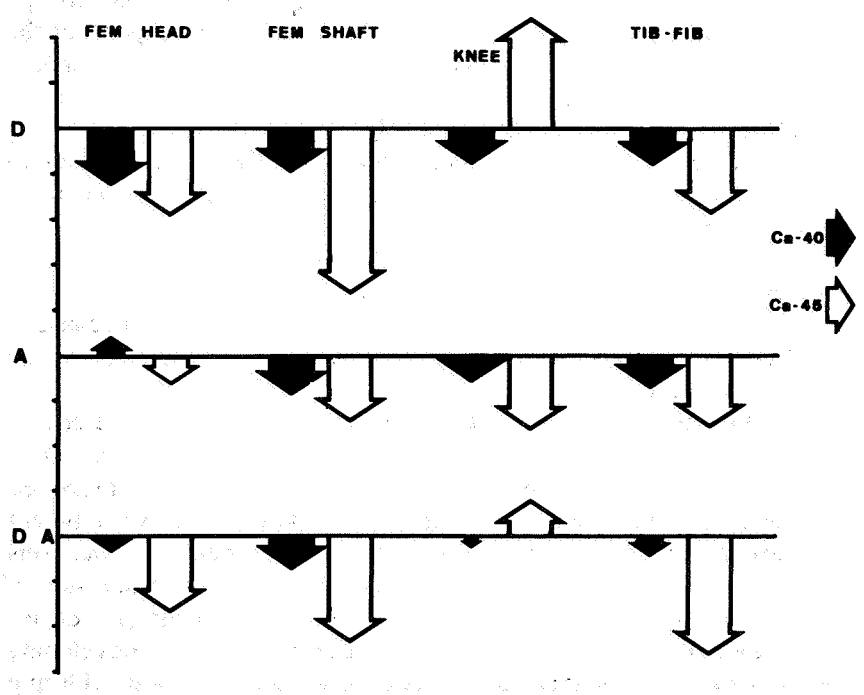

FIGURE 44. Changes in total calcium and in ^{45}Ca uptake in femoral head, femoral shaft, knee, and tibia-fibula of rats after denervation (D), arterial ligation (A), or denervation and arterial ligation (DA).

Over the period of our study, which in this case was during the 10 days following nerve section, there was a fall of between 8 and 15 percent in the total calcium content of the bones concerned as compared with the content of control bones on the other side in the same animal.

But in each case, with one exception, the ^{45}Ca uptake fell even more than did the total calcium content, so that a decrease in new bone formation may have contributed somewhat to the development of the osteoporosis. I will not go into this, but I want to point out that the

knee was the striking exception. Although these epiphyseal plates were fairly inactive they were, as we all know, not fused; the primary spongiosa, the zone of provisional calcification and so forth, were on the shaft side of where we split them, so that all one had here were the epiphyses themselves. Consistently over 2 years in about 30 groups of 5 rats each, we very reproducibly found this considerable elevation in uptake of radioactive calcium by the bone in the knee area, which is quite different from the effect through the rest of the leg. The knee, too, developed disuse osteoporosis at perhaps a slightly slower rate than the other area, but it did so at an elevated rate of new bone formation, whereas the others did so at a depressed rate of bone formation.

MACDONALD: What was the time interval between isotope administration and denervation?

HEANEY: We studied various permutations and combinations with this. We did some 3 or 4 days after denervation and others as long as 10 to 12 days after denervation. Then we sacrificed animals at various intervals from 1 hour to 30 days after isotope injection. This is only one representative schematization of the type of result. Usually, the ratio of the isotope uptake of the operative side versus the normal side was constant throughout the period of study.

I would rather not go into too much detail about how you get disuse osteoporosis, which is another topic entirely. I cite this experiment simply as an example to show that the response of the bone of the epiphyses to the hormonal and mechanical requirement of that leg was different from the response of bone above and the bone below it.

NICHOLS: This was epiphysis per se and not metaphysis?

HEANEY: This was entirely epiphysis. If you attempt to snap these epiphyses off, they come off quite readily and quite reproducibly, and the active zone of cartilage calcification goes into the shaft side, so it was nothing but isolated epiphysis.

RAISZ: Denervation is not the same as disuse. I wonder if the bones were hypertrophic in part because of trauma to the denervated joint.

HEANEY: They undoubtedly did undergo some trauma, but it is difficult to say exactly how much or what effect this had.

COPP: I would like to comment on this point. Shim et al. (ref. 80) also denervated the hind limb in rabbits and observed an increase in bone blood flow in the foot, although there was no significant change in bone mass.

HEANEY: The second section of our experiment was to do the same thing with as complete a ligation of the arterial supply to that same leg as we could get. In this case it was done with arterial ligation alone. There is obviously room for collateral circulation, and this plainly did occur. We controlled this circulation by some postmortem injections of Micropaque into the aorta and took X-rays so that we

could see what the vascular pattern looked like. There was blood flow into the limb, but it came in through collaterals; we could see retrograde flow back up the femoral arteries to the point of ligation, and the ligations remained intact.

There was always a great deal more barium on the intact side than on the ligated side, but there was blood flow there.

With decreased blood supply there was decreased ^{45}Ca uptake in all segments, again repeated at varying intervals. Curiously, the femoral head region gained bone and became, if you will, slightly sclerotic, whereas the other regions became somewhat osteoporotic. When we combined arterial ligations with denervation we obtained the results shown at the bottom of figure 44. The knee region again showed increased radiocalcium uptake despite decreased overall blood flow and almost no change in the bone mass itself.

HOWELL: By those nerve ligations you released tension communicated to the epiphysis as muscle tone. Did you also study the effect of cutting ligamentous insertions about the knee, inasmuch as these would retain a mechanical stress at points immediately adjacent on the epiphysis?

HEANEY: We did not in these animals. We performed another experiment in rabbits by cutting the heel cord, which may or may not be what you are getting at.

HOWELL: I thought perhaps the stress remained applicable to the epiphysis because of the attached ligaments.

HEANEY: These bones also became atrophic, Dr. Howell. They lost at essentially the same rate as the shafts above and below them. Ultimately, if you carry the experiment out long enough, bone loss increases to 20, 30, or 40 percent.

Again, I only want to point out that one geographic region of this animal's leg bone responded differently from another geographic region; therefore, there are many factors which may be invoked that one can think of to explain differences. It is precisely some of these factors that we will be discussing, so I would rather not beg the question.

Dr. Talmage, you have some observations which might fit in now with respect to differences in local bone responses to systemic stimuli.

TALMAGE: It is obvious that everything in our figures will point to effects of parathyroid hormone. However, in line with Dr. Heaney's request, we will temporarily ignore the parathyroid implication and concentrate on the differences in response of two different parts of rat bone, the metaphysis and the shaft of the femur, in regard to the various parameters studied. For our studies, the metaphysis begins at the epiphyseal line moving proximally. The shaft is composed almost entirely of compact bone.

NICHOLS: Is the epiphysis included?

TALMAGE: Only the epiphyseal line—the cartilage is cut off as cleanly as possible. We are trying to deal only with bone; we feel we have succeeded. I would like to reemphasize the fact that there is a very marked difference in the level of equilibration when metaphyseal bone and diaphyseal bone are incubated in serum. Figure 45 is a graph which shows that whether one starts with low or high calcium in the incubating medium, metaphyseal bone produces a higher calcium level in the medium than does diaphyseal bone. This difference can be further exaggerated by incubating the same portions of the femur taken from animals nephrectomized 24 hours before use. In bone from such animals, the equilibration level for the metaphysis is at an even higher calcium concentration, while that for the diaphysis is essentially the same. With normal animals, the phosphate levels in the medium after 4 hours of incubation follow those of calcium.

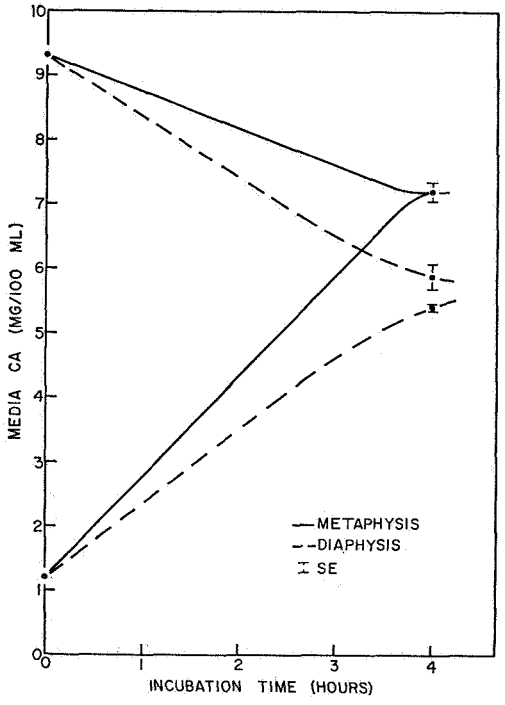

FIGURE 45. Incubation of metaphyseal versus diaphyseal bone in media with normal and low initial calcium levels. Metaphysis differs from diaphysis by $p < 0.005$ at hour 4. [From Cooper et al., ref. 81; reprinted by permission of the publisher.]

These are also higher in the serum after metaphyseal equilibration if bones from nephrectomized animals are used. Since both calcium and phosphate are raised, this indicates a change in the solubility of the calcium phosphate complexes in the metaphysis due to nephrectomy.

PRITCHARD: Is this live bone or dead bone?

TALMAGE: These are live bone incubations; however, the results utilizing dead bone are the same, except that the 4-hour levels are slightly lower in all cases (ref. 81).

The difference in the production of organic acid by these same two areas of bone is given in table IV. Per milligram of bone collagen, there is much more production of citric acid in the diaphysis than in the metaphysis at the end of the bone-serum incubation. Remember, this is per milligram of collagen. Since there are less cells per milligram of collagen in the diaphysis, the differences between the two areas of bone would be greater should the results be based on citric acid produced per cell.

TABLE IV

INCUBATION OF RAT FEMUR IN SERUM [a]

	Metaphysis	Shaft
Citric acid production, mg produced/g bone collagen.....	1.3	[b] 3.0
^{45}Ca removal, following 2-week incorporation, cpm/ml serum...	523	[b] 400

[a] Incubation period was 4 hours.

[b] Values after incubation of bones from parathyroidectomized rats in serum from control rats were significantly less than that for bone taken from parathyroid-intact animals.

A comparison of the amount of radiocalcium removed from these two areas of bone during incubation is also listed in table IV. In this particular experiment, the radiocalcium had been in the animal for 2 weeks prior to sacrifice. There is still more radioactivity removed from the metaphysis than from diaphyseal bone. However, if I may bring in the parathyroid hormone again briefly, it is in the diaphysis and not in the metaphysis that the effects of the hormone can be seen in regard to radiocalcium removal.

Figure 46 shows differences in the effect of the hormone on the rate of incorporation of ^{3}H-cytidine into RNA extracted from metaphyseal and diaphyseal bone after incubation in serum. After 20 minutes of stimulation by a calcium-free peritoneal lavage, a procedure for increasing endogenous parathyroid secretion, there are opposite effects in the two types of bone. There is an increase in uptake in the me-

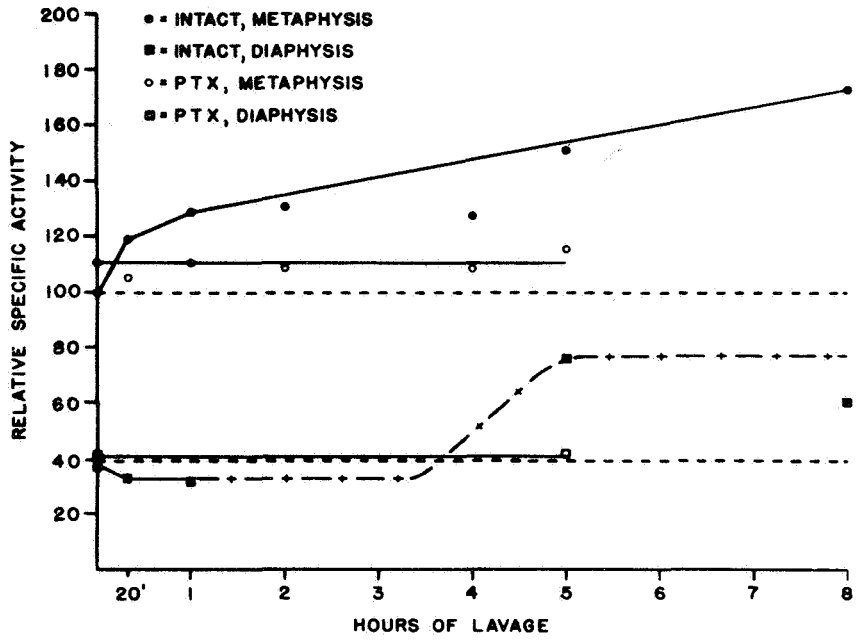

FIGURE 46. Incorporation of ^{3}H-cytidine into RNA. Control metaphysis is expressed as 100.

taphysis and a decrease in the diaphysis. After 4 or more hours of continuous stimulation, the incorporation of ^{3}H-cytidine is increased in both types of bone. I will refer to these figures again in the discussion of cell modulation. They are introduced here to illustrate another difference between these two areas of the femur in the rat.

Figure 47 illustrates the uptake of ^{3}H-thymidine into DNA. The primary difference between the two areas of bone is limited to the greater response in metaphyseal bone.

In summary, then, there are major metabolic differences between metaphyseal and diaphyseal bone, as might be expected. However, they also respond quite differently to stimulation by parathyroid hormone; in the case of incorporation of ^{3}H-cytidine into RNA, for at least a limited time, opposite effects were produced. Obviously, therefore, it is important to ascertain just what type of bone is being studied when using endogenous or exogenous parathyroid hormone, since opposite effects may occur in different parts of bone. This may be just as true in regard to other hormones.

HOLTZER: How many nonbone cells are involved in this?

TALMAGE: How many nonbone cells?

HOLTZER: Out of the total population—marrow cells.

TALMAGE: Marrow cells, leukocytes, and the like? In our incuba-

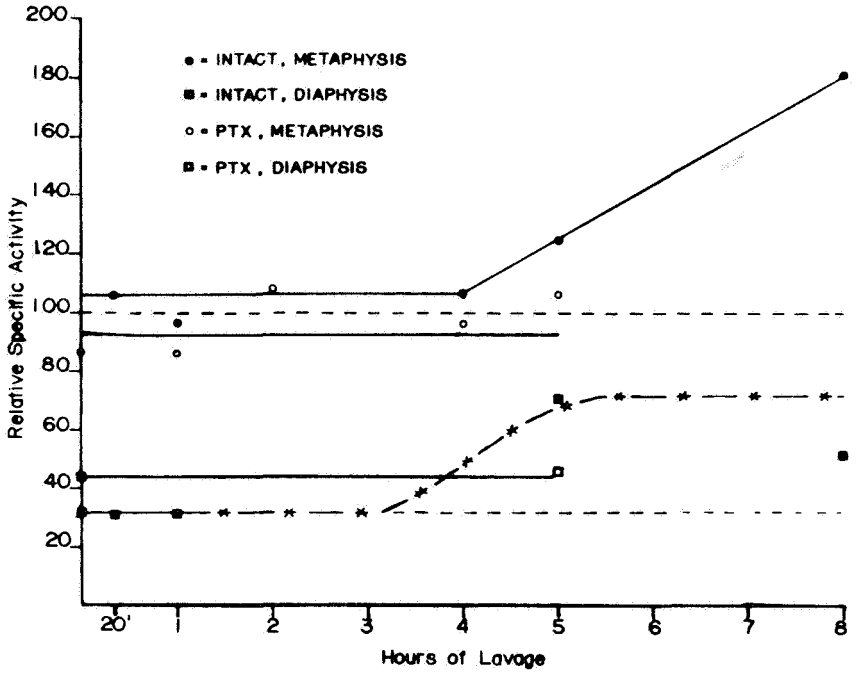

FIGURE 47. Incorporation of ^{3}H-thymidine into DNA. Control metaphysis is expressed as 100.

tion procedures, we wash out the marrow cells before the bone chips are incubated. This washing procedure must be very gentle, as the osteoclasts will not stand much stress and are easily destroyed. We believe we get rid of most of the marrow cells, leaving some leukocytes and those red cells located in the sinuses of the metaphyseal bone.

HOLTZER: There is a large number of vascular cells, tissue cells?

TALMAGE: Yes.

HOLTZER: So this kind of measurement, while certainly suggestive, would be more or less difficult to interpret, would it not, just on the basis of the response of the osteocytes and osteoblasts in the system?

TALMAGE: I did not want to include the osteocytes in this——

HOLTZER: But you are talking about bone—although, in fact, you have a lot of nonbone cells in the system.

TALMAGE: Obviously we have nonbone cells left in this system; there are probably more in the metaphysis than in the diaphysis, because many of these cells are trapped in trabecular bone. However, we are studying parathyroid effects. As yet, this hormone has not been accused of affecting blood cells; so we cannot see how the nonbone cells can be important in our discussion.

RAISZ: The physical difference in these two kinds of bone is very

striking, and it seems to me for that reason that the preparation method is going to affect the way the cells come out. Would you give us more details on how the material is obtained for the biochemical studies that you do?

HEANEY: Are you referring to the cytidine and thymidine studies, or the prior studies?

RAISZ: The citrate and the thymidine studies.

TALMAGE: When the animal is killed, the femur is removed and broken at the epiphyseal line. The cartilage is cut off from the femur with a razor blade. The bone is then cut to separate the metaphysis from the diaphysis. Each portion is gently washed out twice with saline from an eye dropper. Each section is broken into several fragments and placed in the incubation fluid. The total time taken for the entire procedure is 8 minutes.

RAISZ: The diaphysis is simply cut up into fragments?

TALMAGE: And also the metaphysis.

RAISZ: There is a large number of cells inside the diaphysis whose access to the medium is fairly distant.

TALMAGE: That is right.

RAISZ: Whereas there is not such a large number in the metaphysis. Is that correct?

TALMAGE: In distance, yes. But I do not feel that the fact that the osteocyte is away from the surface inhibits it from receiving nutrient. I think there is a very rapid transport into the osteocyte.

NICHOLS: Dr. Owen and I can support that. Within a 4-hour incubation, Dr. Owen, by autoradiography, was able to show proline within the osteocytes in fragments of bone incubated *in vitro* with radioproline in a fashion similar to Dr. Talmage's.

RAISZ: You are studying rates, and as long as you are studying rates, the differences here must be pertinent. I agree that things get in and out, but I cannot believe that there will not be a difference between the rate with which they get in and out through lacunar channels of various sorts, and the rate directly from the medium to the cell. I think these rates are critical to the way in which your system is going to behave metabolically.

NICHOLS: No one will disagree with you about that. As far as I know, however, no one has done a careful study to see how soon the label appears in the surface cells compared with those in the depths, unless you have, Dr. Owen?

OWEN: No; not in tissue culture.

TALMAGE: In tissue culture of embryonic mouse radii, every cell had tritiated glycine in it after one-half hour of incubation. Of course, the distance here was only a few microns.

RAISZ: That is why we use embryonic tissue. We cannot get

a late, highly mineralized embryo to do the things we can get early embryonic bone to do. We have assumed that this was because highly mineralized tissue was not well nourished in the absence of a pulsating circulation which increased both perfusion and mixing.

TALMAGE: I would like to point out that in the incubation procedure there may be less contact with osteocytes than with surface cells. If this is true, then it would even further exaggerate the differences demonstrated between the diaphysis and the metaphysis.

RAISZ: I do not know in which direction we are going.

TALMAGE: The differences are there already, and if we increased the ease of communication with the osteocytes we simply further exaggerate these differences.

PECK: I think the differences that you pointed out are fascinating. I think it would be equally interesting to know what the pool sizes of stable precursors were in the bones at the start of incubation; for example, the pool of stable cytidine with which the radioactive labeled cytidine would mix. You could explain the differences in incorporation between the two bones merely on the basis of isotope dilution if there were large differences in pool sizes of stable material. Do you have any information about this?

TALMAGE: No; but I would agree that if there were large differences in pool size you would have dilution effects. However, could this not still be a transport problem?

PECK: Particularly, as has been suggested, if there were different cell types.

PRITCHARD: Is the cytidine going to new RNA or exchanging with old RNA?

TALMAGE: Unless my information is wrong, the cytidine goes first into a new messenger RNA.

HOLTZER: That does not follow. It is not true that all of the rapidly incorporated cytidine and uridine must go into messenger RNA. Any autoradiographic study, whether with an ameba or with bone cell, suggests incorporation first into the nucleolus and later into the cytoplasm. There is now much evidence which suggests that the nucleolus is responsible for synthesizing ribosomal RNA. Where and when messenger RNA is made in tissue cells is by no means clear.

But I agree that uridine and proline will penetrate into tissues several millimeters in thickness. In less than 5 minutes these isotopes will penetrate into a piece of cartilage over 2 millimeters in thickness (ref. 82). But I would emphasize, again, that it would be interesting to know exactly which cells are responsible for all the activity you are describing.

TALMAGE: Naturally we are attempting to do that. We have separated out a fairly pure preparation of osteoclasts. Also, we

have a mixed preparation of mesenchyme cells and osteoblasts, and we hope very shortly to have a pure preparation of osteocytes. We are working on it.

RAISZ: I think it should be pointed out that embryonic cartilage is quite different from bone with respect to diffusion between cells.

HOLTZER: Is there any evidence for that? How can you——

RAISZ: Polarize tissue?

HOLTZER: Yes; but is there anything more charged than, say, chondroitin sulfate in terms of exclusion? I would not have thought that only on the basis of its charge, cartilage would be more difficult to penetrate than bone in terms of an ion-exchange kind of effect.

RAISZ: But not in terms of water content.

ARNAUD: I would like to know whether or not any of the studies done up to this point have been carried out in grossly marrow-contaminated bone and whether or not the results were different.

NICHOLS: Can I speak on this point, because I think it is a critical one? I will come back to it again when I show you metabolic data about marrow and bone cells derived from the same bone sample.

The whole problem of the importance of marrow contamination came up some time ago. It turned out that the percentage of the total cells which are demonstrably marrow is not terribly large, but there is no good way of measuring this precisely; the metabolic patterns of the marrow cells are somewhat different, and the total metabolic activity tends to be less than that which we see in the bone. Therefore, the fraction of the metabolic activity that can be attributed to contamination is probably quite small, 10 percent or so.

With respect to the problem of the different pool sizes into which RNA precursors are assimilated, we have some data that relate to the cellularity of the tissue and therefore indirectly to pool size. If one plots the DNA or RNA content per weight of different kinds of bone against age in months of rats, one gets curves like those in figure 48. The age groups range from 3 weeks (the minimum age at which I felt I could separate metaphyseal, trabecular, and cortical bone clearly) to 22 months (the age at which half the animals had died). The curves for DNA show clearly that the cellularity of the two types of bone is quite different—cortical bone being, of course, always the lesser of the two. More important, however, is that while the cell content of cortical bone decreases steadily with age, the cell content of trabecular bone actually rises up to the age of 4 months and then falls abruptly.

Although the RNA data only extend to 8-month-old rats, they follow similar patterns. Again, the RNA content of cortical bone falls rapidly with advancing age up to 4 months (the point where growth has leveled off), and then declines rather slowly. RNA in trabecular bone starts lower than in the cortex but remains stable through the

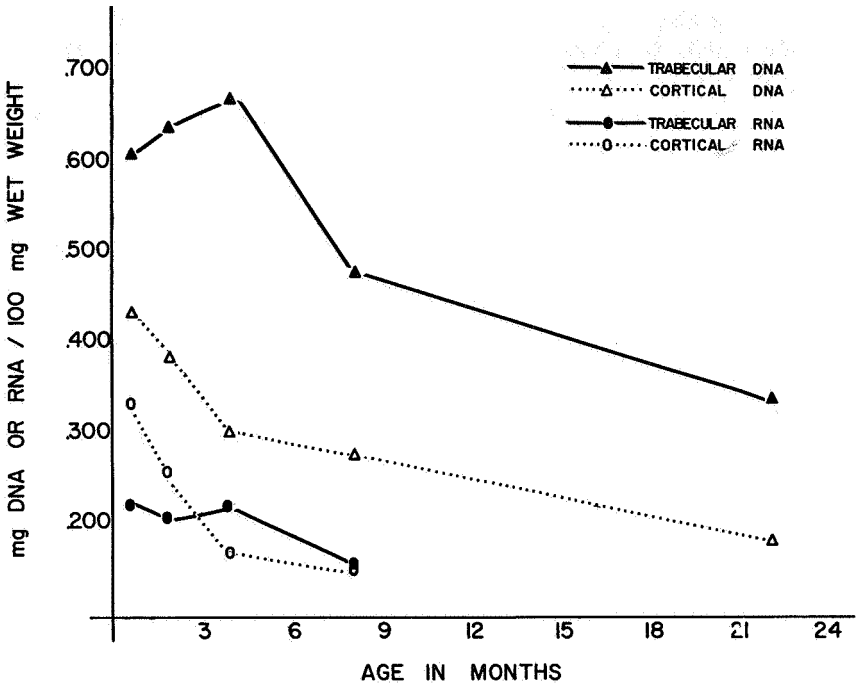

FIGURE 48. Content of DNA and RNA per weight of trabecular and cortical bone of rats.

growth period only decreasing after 4 months of age. These data show that there is indeed a considerable difference in pool size with differences in age and in bone type. Therefore, unless the pool size is known, your observations—while interesting and provocative—cannot really be interpreted.

HOLTZER: Are you calling cells the pool size?

NICHOLS: Yes; in the sense that the pools are in the cells, and hence the overall size of one is related at least indirectly to the size of the other.

I have done one other experiment regarding cells and their activities that might be of interest in this discussion. This experiment was performed only once, and I am not sure how quantitative it is. However, if one takes chips of bone and separates the cells from them by grinding in a mortar with successive batches of medium—the way we do (each batch is poured off, the fragments of calcified collagen are allowed to settle for 30 minutes, and the cells are finally harvested from the resulting supernatant by centrifugation)—one obtains a progressively smaller number of cells with each successive grinding, until all the fragments have been ground away.

Thinking about this procedure, it occurred to me that as one grinds, the first cells to come off are probably mostly marrow. The next are probably surface cells, while the later batches contain larger and larger proportions of osteocytes, until the final ones should contain virtually nothing else.

It turned out that when the cells in each of the five successive grinds were examined, there was a progressive difference in their metabolic patterns. Very simply, it seemed that as the osteocyte is approached, the oxygen consumption per milligram of cell DNA goes down to virtually zero, and the lactate production, very reasonably, goes up. Moreover, looking at a smear of the cells under a light microscope, one finds that the first grinds contain mostly round mononuclear cells with moderate amounts of cytoplasm, while the last ones are elongated with scanty cytoplasm and a nucleus which seems to bulge the cells in the middle. Now, whether these are really osteocytes, I do not know, and I do not know what their electron micrographs look like; I only say I think they might be osteocytes.

HOWELL: Is that from cortical bone?

NICHOLS: Yes; from rats about 50 days old.

PECK: One important point about the problem of working with whole tissues and individual cells *in vitro* is the possibility of leakage of cytoplasmic enzymes (ref. 83). Some tissues, in which cells are encased, may provide greater protection against loss of enzymes than other tissues, in which the cells are widely exposed to the bathing medium; compare, for example, diaphysis with metaphysis or whole bone segments with isolated cells.

Dr. Talmage, it would be interesting to study the relative appearance of enzymes in the medium during *in vitro* incubation of metaphyseal and diaphyseal bone; for instance, those enzymes associated with citrate metabolism.

PRITCHARD: When bone is grown *in vitro,* the cells walk out one by one. The first cells that emerge do not have much phosphatase in them, but the second wave shows normal phosphatase activity. Could you not get a useful separation of cells just by letting them wander out under their own steam *in vitro*?

NICHOLS: There you get into the problem, do you not, of possible dedifferentiation as the walk starts.

PRITCHARD: Yes, I suppose so; but it is worth a try.

HEANEY: From listening to this discussion I have the impression that some of the differences Dr. Talmage has cited might be a result of artifacts in the study method rather than a result of the local factors with which I am concerned; therefore, I would like to return to our topic lest it evaporate entirely.

A number of years ago, Dr. McLean and his collaborators published

some experiments on the effect of parathyroidectomy on haversian bone remodeling (ref. 84). I know that he has done some additional work in this field since this publication and that this work is in keeping with our topic of excluding hormones, rather than including them.

MCLEAN: Would you like me to introduce the subject of internal remodeling?

HEANEY: Please.

MCLEAN: I am not equipped to give a didactic lecture at this time, but I have some figures to present. I would like to recapitulate, particularly for the benefit of those who are not thoroughly familiar with this subject, what goes on in internal remodeling.

In 1853, Tomes and De Morgan (ref. 85) described what they called absorption in bones. They showed that the cavities assume the form of tunnels, of which figure 49 is a cross section taken from Petersen (ref. 86); that when absorption was going on, these tunnels were lined

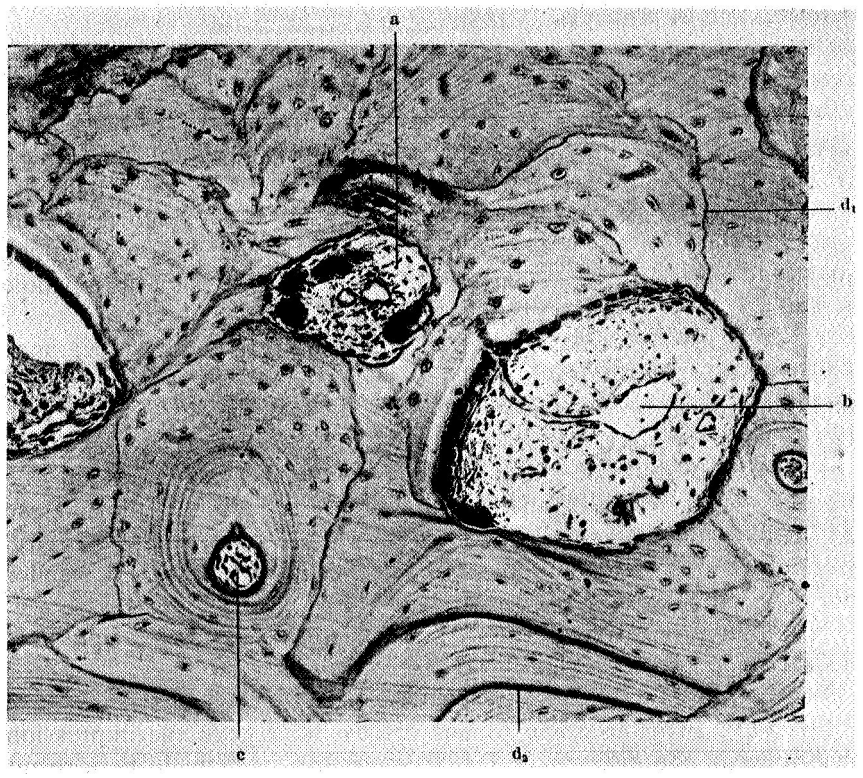

FIGURE 49. Ground section of human bone showing formation of osteons. During absorption, tunnel lined with osteoclasts, *a*; reversal of process during the filling in of the tunnel by osteoblasts, *b*; tunnel filled in by the deposition of successive lamellae, *c*, with a haversian canal. [From ref. 86; reprinted by permission of the publisher.]

with osteoclasts; and that when they reached a certain size, fairly uniform for an individual, the whole process was reversed, and instead of osteoclasts, the tunnel became lined with osteoblasts.

Tomes and De Morgan made a very astute remark about this. They said that as far as they knew, the same cells that accompanied the resorption of bone were not building new bone. I think we still believe that these are the same cells, which reverse their functions, change their form, and start making new bone.

HEANEY: There is some disagreement with that comment, Dr. McLean; shall we postpone that discussion for now?

MCLEAN: Yes. To continue, the tunnel is now lined with osteoblasts, and it is filled in by the deposition of successive lamellae, everything moving toward the center, so that eventually we come down to the haversian canal that was shown previously in Dr. Robinson's material.

Dr. Robinson also showed a narrow band of uncalcified tissue; he referred to it as prebone. I prefer to use the term "preosseous tissue," or it may be called osteoid. There is a very thin layer of uncalcified matrix, mainly collagen and ground substance; immediately around this layer is the calcification front (shown in previous electron micrographs), the little rough areas where new mineralization takes place.

We have then, in a working osteon, several morphologic landmarks. First, there is the lumen of the osteon, which contains the blood vessels and whatever else is carried within the haversian canal; around the lumen is a thin layer of uncalcified tissue; then there is a layer of very reactive tissue. I will show in a moment what goes on in the reactive tissue. Everything beyond the thin layer is calcified. This area calcifies very quickly, up to something like 70 percent of its final mineral content within a matter of days; it may take weeks to add the other 30 percent.

The particular point that I want to emphasize is that the reactivity in this osteon is almost entirely limited to the calcification front. The uncalcified osteoid zone is not reactive, except that it will take up sulfated mucopolysaccharides. They are part of this structure and they are incorporated within this layer. But this layer is not yet calcifiable; suddenly it becomes calcifiable and the calcification process moves in.

Concentric lamellae are formed until the canal finally narrows down to its ultimate dimension, averaging approximately 20 microns in diameter, and then all new growth ceases. There is no longer a zone of preosseous tissue; there is no more mineralization. Mineralization has been completed, and the entire structure then becomes a resting osteon.

Some people disagree with this characterization, but Jacques Vincent

introduced the terms "metabolic" and "structural" bone. In his terms, the metabolic bone is at the margin of the calcified area, and I think he had some idea, also, that the partially calcified osteon is reactive until mineralization is complete. This work was subsequently published by Vincent and Haumont in 1960 (ref. 87).

This sequence is illustrated in table V (ref. 88) which lists five zones. Zone A is the haversian canal lined with osteoblasts. It can be stained with histologic stains, but there is no particular reactivity in the membrane of the canal, and there is nothing different in these osteoblasts from those in other parts of the skeleton. Zone B is the preosseous tissue, which Frost and Villanueva (ref. 89) have called the osteoid seam. This can be seen with histologic stains, and it takes up radiosulfate *in vivo;* so that if one gives ^{35}S to an animal, some of it will be deposited in this zone.

Zone C contains all the activity. This calcification line (calcification front or calcification zone) will take up ^{45}Ca, radium, strontium, phosphorus, cobalt, lead, tetracycline, and alizarin, all in the same place. This is also the zone where zinc is deposited in the normal metabolism of the animal. If radioactive zinc is given, it is also deposited in this zone. Cadmium competes *in vivo* with zinc; both appear to react as trace metals which catalyze some reaction and possibly involve catalysis of mineralization. This zone takes up Sudan Black

TABLE V

CROSS SECTION OF FORMING OSTEON, FROM CENTER TO PERIPHERY

Zone	Description	Reacts with—
A.....	Haversian canal, lined with osteoblasts and housing blood vessels, nerves, lymphatics, and connective tissue	Histologic stains
B.....	Uncalcified preosseous tissue (osteoid seam)	Histologic stains, ^{35}S *in vivo*
C.....	Calcification line (calcification front)	Mineral component, reacts with ^{45}Ca, ^{226}Ra, ^{90}Sr,^{32}P, Co, Pb, tetracycline, alizarin, etc.
		Organic component, reacts with ^{65}Zn *in vivo* (Zn also demonstrable in untreated animal), Cd (competes *in vivo* with Zn), Co, Sudan Black, and other stains
D.....	Partially calcified osteon.....	^{45}Ca (after *in vivo* or *in vitro* pretreatment with acid)
E.....	Cement line (external limit of osteon)..	Histologic stains

very specifically, and some other stains also. Therefore, this is the reactive portion of the osteon and accounts for nearly all of the reactivity.

Zone D is the partially calcified zone, and zone E is the cement line, the external limit of the osteon.

Figure 50 is a beautiful illustration from Leblond and Lacroix (ref. 90). Section A of the figure is the histologic section; you can see the calcification zone stained with cobalt. This zone also takes up Sudan Black. Next to it is the uncalcified zone, which is not reactive and does not stain with either cobalt or Sudan Black.

Section B is the microradiograph with arrows to localize the zones so that one can compare the histologic section with the microradiograph. Note that the preosseous tissue does not stain and casts no shadow in the microradiograph.

My particular point is that there are two things that give life to bone, and bone—far from being an inert structure with purely structural functions—has a great deal of life in it. One thing that gives life to bone is the osteocyte; it will be discussed further so I will not dwell on it.

I would like to go on for a moment with the idea that internal remodeling, which means the formation of new osteons, goes on throughout life. We used to say it went on throughout life but at a diminished rate with increasing age. Now we have decided that, if anything, remodeling is more active as the individual ages.

The reactive zone in the osteon forms a continuum between the fluid containing calcium and the calcium in the solid form, so that there is a line of demarcation, which was shown clearly in the electron micrographs; this line of demarcation constitutes the bridge between the fluid-state and the solid-state mineral. Whether this bridge is to be regarded as a solution of calcium already in combination with phosphorus or not is not clear; but it is evident that this line is the border zone, and it does afford a bridge from the fluid state of the solution of the mineral in the blood and the tissue fluid to the solid state in the bone.

It is my belief that this process is essential to life and that what we have been describing is essential to homeostasis and to the maintenance of a constant calcium concentration. It should be noted that we have described a one-way process; i.e., the mineral going into the bone. That is very easy to demonstrate. Whether mineral can also come out of this complex into the blood is something that still remains to be shown. I do not think we need to postulate that this mechanism has anything to do with the passage of mineral back into the blood. We have two excellent mechanisms for that, one with the osteoclasts and one with the osteocytes, so that the needs of the body for mineral

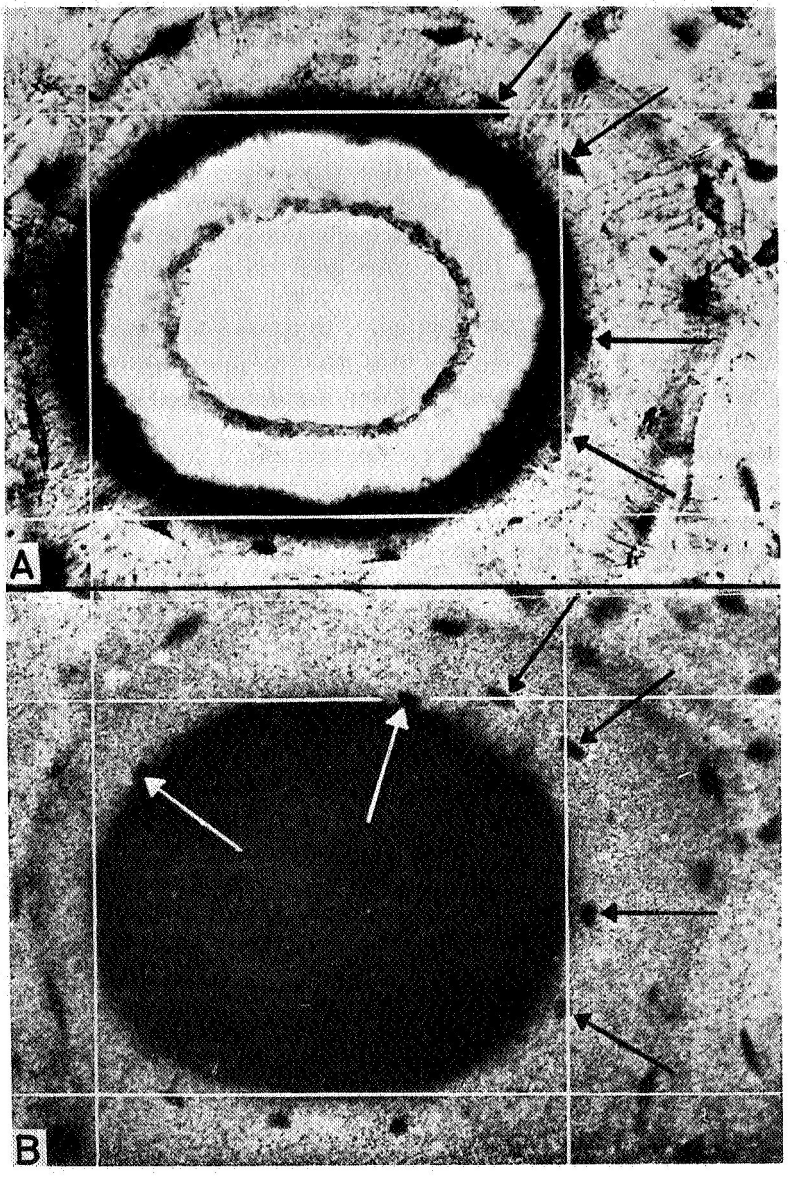

FIGURE 50. (*a*) Undecalcified ground section of compact bone treated with cobalt nitrate. (*b*) Microradiograph of the same section. Arrows indicate zones. 533 ×. (See table V.) [From ref. 90; reprinted by permission of the publisher.]

to be returned to the circulation are taken care of by cells other than those in the osteon. But it is still true that the osteon is important

in the overall balance between mineral deposition and demineralization or resorption.

Several years ago with Jenifer Jowsey, we did some experiments on dogs to determine whether this resorption process in bone is under the control of the parathyroids (ref. 84). That question is still, I think, unanswered, but to my mind, at least, the weight of the evidence so far is that this process is not under parathyroid control. It goes on even in the absence of the parathyroid glands just as growth goes on. The remodeling incident to growth also goes on in the absence of the parathyroid glands.

Now, there is one more question. What triggers this mechanism? Amprino (ref. 91) gave me the idea that the particular locations, the areas in which new osteons will form, are determined by the stresses and strains on the bones. The amount and location of bone tissue undergoing resorption seem to be controlled by mechanical conditions characteristic for each skeletal location. Once the areas of skeletal tissue to be remodeled are determined by mechanical factors, then the actual location of the individual tunnels is apparently a random process. No one has offered any explanation as to what starts one of these tunnels forming or as to what determines a particular location for any of these tunnels. However, bone remodeling, whose first phase is resorption, comes into play when liberation of mineral from bone is not sufficient to meet the physiologic demand.

HEANEY: I think one could disagree with that because, although the specific localization might be random, the polarization is such that it could not be random, could it?

MCLEAN: I think it is true that tunnels never go across the bones. In the long bones they go in the long axis of the bone, but not precisely. As you said a while ago, they have a slight spiral turn.

URIST: Do you refer to the work of Amprino on stresses and strains after an experimental fracture?

MCLEAN: No.

URIST: How did he do that experiment? Has it been published?

MCLEAN: Amprino (refs. 91 and 92) himself referred to his postulates as assumptions, and stated that further research is needed to support them.

URIST: It is easy to demonstrate the bone deposit on the concave side as a response altered stress after a fracture, but it is more difficult to demonstrate the osteogenic response to stress on an unbroken bone. I would like to see someone put stress on an unbroken bone with some kind of apparatus and show that the resorption cavities begin to form chiefly on the side of the strain.

This also brings up another question. Let us assume that one makes cross sections of the bone and that where the bone is bowed, on the

convex side, there are more resorption cavities than on the concave side. Let us assume that one makes a series of cross sections, maps this out, and makes careful counts of resorption cavities. The question is, Is the difference the cause or the result of the bowing?

HEANEY: It is impossible, offhand, to answer that question; it is easier to ask it than to give an answer. I might cite an observation reported by Putschar (ref. 93) and quoted by Lent Johnson (ref. 94) in his encyclopedic chapter in the Henry Ford Hospital Symposium. Putschar observed a young adult who had practically complete poliomyelitic paralysis of both legs acquired at 1 year of age and showed subnormal osteonic remodeling; he stated that this certainly suggests that mechanical, in addition to genetic and metabolic, factors play an important part in the formation of cortical osteons.

PRITCHARD: I do not think anyone has much information on this point, but it is known that remodeling is very intense at the attachment of very powerful tendons, for example, at the linea aspera of the femur.

Then, of course, there is the work on the remodeling of vertebrae in scoliosis, and on the movement of tooth sockets with orthodontic appliances, in which a close linkup has been established between the known stresses and strains and the remodeling reactions of the skeleton.

HEANEY: I think we can say, as Dr. McLean has pointed out, that internal remodeling has considerable homeostatic significance, that is, significance for the extracellular fluid calcium homeostasis; but aside from that, remodeling will exist anyhow and is probably independent of parathyroid hormone. Certainly, in its topography it is independent of hormones involving calcium homeostasis. The real question we must ask is, Why is it here? What is it that determines that it is in this place, and that it is at all?

It has taken a while to get around to asking some of the fundamental questions, but I think that we must give some thought to the following questions. Does the aging of the bone material itself somehow exert an influence on subsequent turnover? That is, when bone is formed, does a cycle of absorption get initiated which ultimately predisposes to subsequent removal of the bone itself? What is the relationship between the nutrition of the bone cells and their blood supply? What role, if any, does this relationship play in subsequent removal and reconstruction? Finally, there is the obvious question, which we have all known about for a number of years, concerning the role of the mechanical forces and the electrical effects thereof. Are these mechanical forces, when all is considered, adequate to explain all that we know about and all that must be explained with respect to local factors?

We have not given formal consideration to these questions as yet, but it seems to me that these are some of perhaps many more which could be introduced. It is against this background that I would like

to have Dr. Currey talk, for he has given much thought to such questions. I would like to turn the discussion over to him now.

CURREY: In this gathering I may seem a bit of a heretic because I am not going to talk about mineral metabolism. I am going to talk first about haversian systems, or rather, internal remodeling.

Concerning haversian systems, there are two questions. Why do they form at all? Why do they form in the places they do? In fact, these two questions always get congealed.

People who have thought about haversian systems have produced about four reasons as to why they should be formed. One reason is that bone becomes necrotic; cells die and the bone is replaced by new, healthy bone. Another reason is that the haversian systems in some way make the bone mechanically a better job than it was before the haversian systems were formed. This was thought to be the case by reasoning from trabeculae. Trabeculae look like haversian systems out in space and are clearly mechanically adaptive. There are haversian systems in bone, and there are trabeculae in bone; both are mechanically a good thing.

Another suggestion was that haversian systems remodel the initial, rather rapidly put down, blood supply and make it more efficient. A final reason is that these systems remodel for mineral metabolism purposes, making new bone when calcium and phosphorus are available and so on, which we have heard about.

I would like to start by discussing the evidence that the exact place in which haversian systems are formed is determined by cell death. If you take a thin section out of bone (preferably cut by saw), fix it, grind it down but do not decalcify, and then stain, you get a very different idea of what bone looks like than if you decalcify it and stain the collagen and soft parts. There is a distinct difference between those parts of the bone where there are no cells, the lacunae are empty, and there are no nuclei showing, and other places where the nuclei stand out strongly.

If you take a young calf and look at its bones, every lacuna is filled with a nice, strong staining nucleus. If you take a man of 75, outside the haversian systems you find a great proportion of the lacunae empty.

I determined the distribution of dead cells and live cells as shown by whether or not there was a sharply stained nucleus, and it was quite apparent at once that inside the haversian system, inside the cement line, usually practically all the cells are alive. By the very nature of things there must be areas outside all haversian systems (interstitial lamellae), and one immediately sees that the proportion of dead cells in those places is much higher than it is inside the haversian system.

The reason for this would seem fairly apparent. There are good

canalicular connections between all lacunae inside the haversian system, but across the cement line very few canaliculi pass; therefore, diffusion is difficult, these cells get cut off from their blood supply, and tend to die.

Now let me draw a contrast between haversian bone, such as is found in humans, where practically all bone is occupied with the haversian system, and what I call laminar bone (what Enlow and Brown (ref. 95) and others have called plexiform bone), such as is found in cattle, where there are a series of two-dimensional, flat networks of blood vessels with fairly large-bore channels connecting them (fig. 51). There is a very good blood supply in the plane of the networks, but the supply is not so good in the plane at right angles. This is very characteristic of large, hollow bones.

Another observation I made when looking at beef femurs is that if one looks at a single lamina, at the midpoint between the two blood vessel nets, there is what is called the bright line across which very few canaliculi pass, rather like across the cement lines in haversian systems. (See fig. 51.) This is not a reversal line. I found in cattle bone that it was rare to find only one haversian system in this primary bone. What one tends to find is several in a row, occupying a single lamina. This suggested to me that if for some reason a haversian system formed in a particular place in this laminar bone, the blood supply of the areas on either side of it in the same lamina could not be made up by blood flowing from the neighboring laminae. Therefore in the middle of this network, there would be a kind of cylinder across which no blood passed. This cylinder would tend to restrict the blood supply on each side. This seemed to be a reasonable explanation of the fact that these haversian systems occur in rows and usually extend up to, but not farther than, the bright line in the middle between two laminae.

So the morphology certainly makes sense if you have one haversian system forming, then you will be liable to have more haversian systems forming around it, simply because the first system interrupted the blood supply locally.

I looked at bone that was very healthy, that is, bone with very few lacunae without strong staining nuclei, but which had a few haversian systems forming and in which the outline of the cavity was irregular; there was no cement line.

I then looked to see if there were any dead cells in about 30 microns around the outside of this forming haversian system cavity; I also looked in comparable areas at random elsewhere in the section. The bone as a whole was very healthy; that is, dead cells were rare. I found that there was a significantly greater number of dead cells in the neighborhood of forming haversian systems than there was in the generality of the bone. The number of dead cells was not very high, but never-

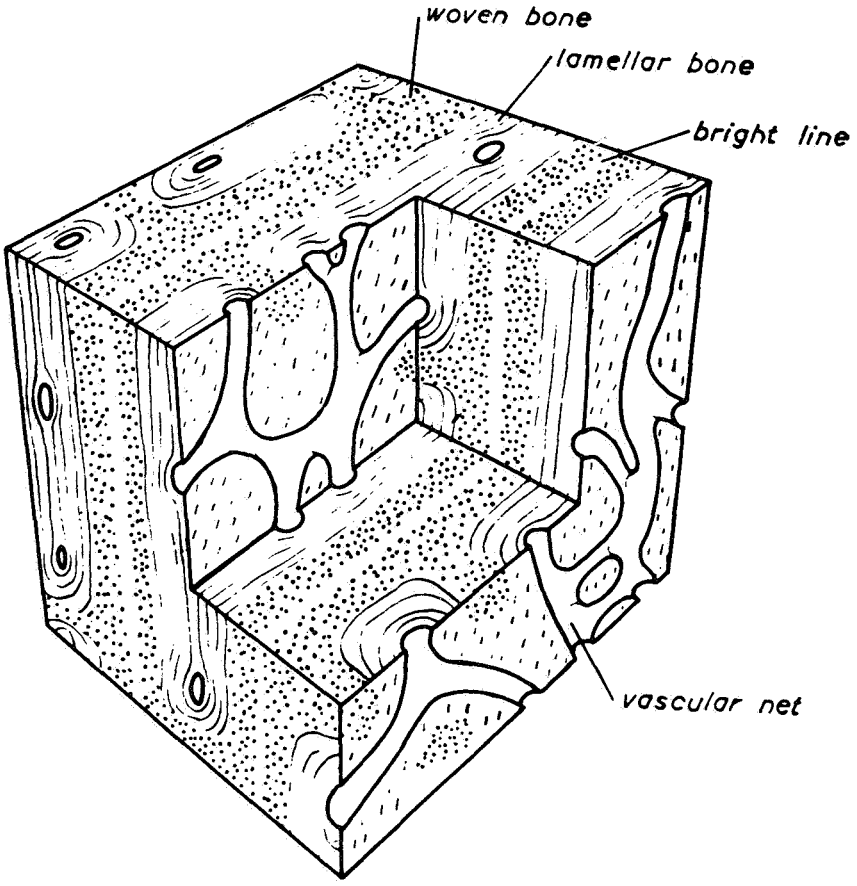

FIGURE 51. Block diagram of a small portion of laminar bone, showing parts of the vascular nets of two laminae.

theless it was significantly in excess around forming haversian systems than elsewhere.

Now, to what does one attribute this?

HEANEY: In what you have said, particularly with respect to the lamina of bone, it has been assumed that a poor vascular supply predisposes to haversian remodeling. Would you care to cite the evidence for this position?

CURREY: No, I would not; I would rely on commonsense. If you have no blood supply and no canalicular connection with neighboring cells, and if you are completely isolated except for what actually gets through the hard bony tissue, then it would seem to me reasonable to expect that these cells would be in metabolically a much less favored place than those cells that were in close connection with blood vessels

to which their canaliculi extended. This would seem to be commonsense. I have no evidence for this.

HEANEY: There is no question that, deprived of a proper blood supply, the cells would be compromised; but the question is, Does this predispose to osteoclastic resorption?

CURREY: Well, I do not know. I am just producing a series of observations which might indicate that where cells are in a bad way is where you tend to get haversian systems. To explain the observation where I looked at the healthy sections with a few dead cells and the dead cells tended to be around forming haversian systems, you might say it was the process of the forming of the haversian system which leads to cell death. This may be so.

I did another slightly finer analysis of this. I looked at rather small, forming haversian systems. Haversian systems have a fairly characteristic size in any part of a bone, and one could say, "Well, that has gone only about a third of the way." One can tell the size it is going to be before it starts. And I found the ones that were very small, that did not get very far, had dead cells surrounding them more frequently than those that had practically reached their full size; therefore, it is as if—and this is pure hypothesis—there is a blood channel in a primary osteon, not in a reconstructed osteon, and a dead cell nearby. This may cause the formation of a haversian system at that level. I may say that the sections studied were fairly thick and include in their length about the distance that Cohen and Harris (ref. 77) found tended to be remodeled at any time. Haversian systems do not form along a great length at one time. I thought it might be reasonable to suppose that where there are one or two dead cells, osteoclasis may in some way be stimulated in that region, and there will tend to be a haversian system formed at that point. Therefore, if you look at the small systems still forming, your chances of finding the dead cells outside the area are higher than if you got to the outermost level, where they would very probably have been resorbed.

It is true that the formation of an erosion cavity does not lead in any general sense to the destruction of cells; I very frequently saw nuclei half in and half out, just before it was incorporated into the central space.

Figure 52 illustrates the very characteristic kind of pattern which one can observe. Figure 52(*a*) is a haversian system with two blood channels, with an erosion cavity starting from one channel. Figure 52(*b*) is a later stage where old cells that were surrounding the central blood channel are now cut off from the new blood supply. The blood supply has gone sideways. You can follow it up for several generations quite frequently (fig. 52(*c*)). The cells were cut off when the new haversian system was formed.

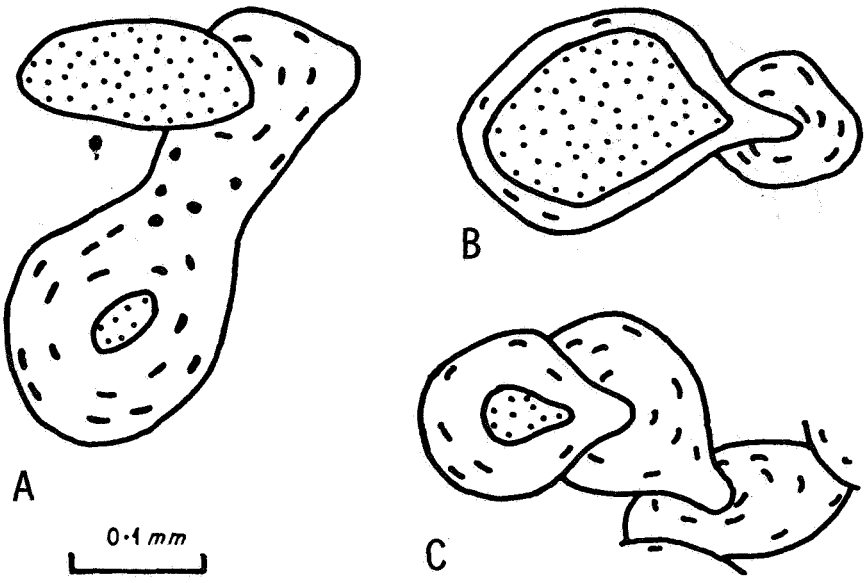

FIGURE 52. Three stages in the formation of haversian systems.

You can see the kind of logic in the morphology. It is apparent that these older haversian systems are going to be in a worse way than the new ones, and this certainly does seem to be the case if you look at the proportion of dead cells inside and outside the haversian system.

You might say, "Well, this is why haversian systems form in one place." It may be that one or two form, and this interrupts the blood supply and the general metabolic setup there; then there are more and more forming.

There are one or two things against this. In figure 53 are drawings of cat ulnae; the stippled area is primary bone, the white area is haversian bone, and the black areas represent forming haversian systems, right and left. The agreement of the places where the haversian systems are forming is rather remarkable. The areas of haversian systems are in almost exactly the same place.

Marotti in 1963 (ref. 96) has done this in more detail and gets practically the same picture. You almost get stereo pairs of the formation of haversian systems which, in this case, are not altering the external form of the bone at all.

Any suggestions as to why one should find haversian systems forming in exactly the same place? I do not think that it is because of the idea I have produced here, that is, interruption of the blood supply. It would be unlikely that one would obtain such a neat agreement.

HOLTZER: Could you do the obvious experiment of putting in something like skull bone as a graft? Do you think these haversian systems

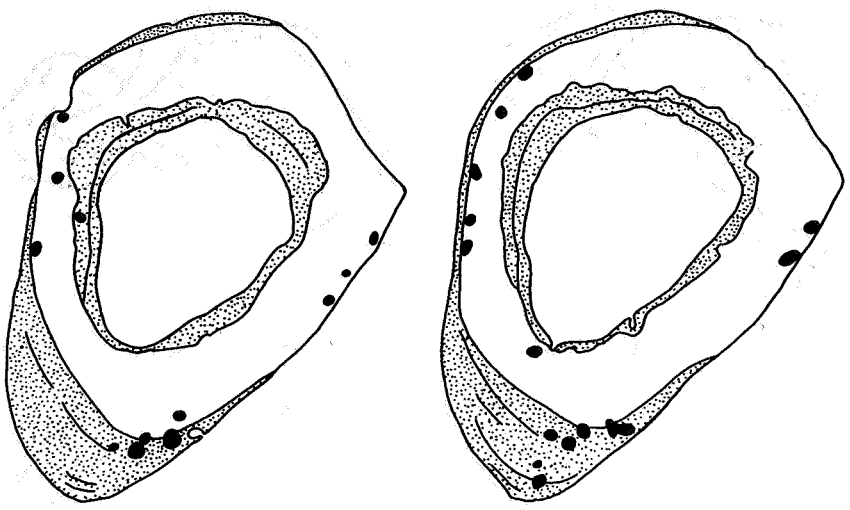

FIGURE 53. Camera lucida drawings of the right and left ulna of a cat. Stippled area is primary bone, white area is haversian bone, and black areas represent forming haversian systems.

would go right through a complex bone that does not have the usual haversian system? I want to know whether it is the whole area that is destined to be canalized by the haversian system, or is it something peculiar about the bone? By putting another kind of bone in there, could you get some information on what causes these deposits?

CURREY: You quite probably could. Of course, the trauma of actually putting in a graft and interrupting the blood supply to put in a graft would probably negate any virtue to it.

HOLTZER: Could you not come back to it 2 years later?

CURREY: Yes, possibly.

URIST: What is the external shape of the bone at the level of the section?

CURREY: Are you talking about the stress on the bone producing the gaps?

URIST: Were there convexities or concavities or neither of these in the three lines of section? Were you able to see anything on the external contour of the bone that would coincide with the localization of resorption cavities or overgrowths?

CURREY: I did not look specifically for that. I think any strain would be too small to be noticeable at this level. The stress would have to be pretty large to produce local humps like this.

PECK: How about insertions?

CURREY: In the linea aspera, for instance, there is much more reconstruction under the insertion. This is even more marked in cattle,

where the homolog of the linea aspera is usually the only place where you get any large amount of reconstruction in the femur.

I took an 8-year-old cow, which was called by the farm "a good milker," because I felt that this cow might be under stress as regards calcium. If there is any stress, those giving a lot of milk might be expected to show quite a lot of haversian remodeling, and there was not a trace of it except under the muscle insertions.

RAISZ: Do they resorb in this laminar area?

CURREY: No; the only way to get resorption in a laminar area is to form a haversian system in the ordinary way.

WHEDON: Have you looked at bones from animals of different ages? Is there a difference with age in the formation of the haversian system?

CURREY: I have looked at young cattle and old cattle; there is no alteration.

WHEDON: The reason I asked this question is that Smith and Walker (ref. 97) have shown that in long bones as they age the cortex becomes thinner, but the actual diameter of the bone increases because the bone is sort of expanding as it ages. One might expect that there would be some indication by one method or another of remodeling along the outer surface rather than along the inner surface.

CURREY: Yes, but I am talking only about reconstruction that takes place entirely within the cortex of the bone. I think what goes on in the surfaces is another matter.

You do get changes in the mode of reconstruction with age. Older people form smaller haversian systems (ref. 98). They have more and smaller haversian systems than younger people.

ROWLAND: Before you go on, may I interrupt for a moment? You referred to the work by Marotti (ref. 96)—he also showed that haversian remodeling occurred in similar locations when pairs of long bones were examined at identical levels. But there is a fourth dimension involved. You are looking at a two-dimensional representation of a three-dimensional section; the fourth dimension is time. Should we not expect that the number of such sites will change as the animal ages?

CURREY: The number of what?

ROWLAND: The number of resorbing areas or rebuilding areas; that the number, the gross number, was a function of the animal's age.

CURREY: Yes.

ROWLAND: Does this not tend to eliminate the possibility that the raw number is a function of stress?

CURREY: If the quality of the bone changes through, for example, increased mineralization so that it becomes stiffer, the bone may have a greater or lesser strain to put up with. I do not know. I do not mean to eliminate this. I am not putting forth the idea of strain particularly. I am not offering any ideas as the only answer. I am utterly confused.

Now I would like to continue and to ask whether haversian systems are a good thing from a mechanical point of view. This is, more or less, the first experiment I did when I started working on bone (ref. 99). I obtained a whole series of samples of beef femurs, tested them in a tensile testing machine, and correlated the strength with the known percentage of haversian systems; the answer was quite straightforward. The more haversian bone, the weaker is the bone in tension.

This work is not being repeated exactly, but a complementary study has been done recently by Heřt et al. (ref. 100); they did not get a nice clear answer such as I did because they employed a different method of plotting. They talked about bone that is almost entirely haversian, on the one hand, and all the rest of the bone, on the other hand, so they have lumped a great part of my distribution. But they certainly did not find that the haversian bone is stronger than primary bone.

Therefore, the idea that haversian remodeling makes a bone stronger in tensile strength is not a good one.

PRITCHARD: It is not a good one? Would you repeat the suggestion that there are a lot of these haversian systems which are not fully calcified and that this is why haversian bone is weaker than other bone?

CURREY: I would not want to repeat this, because at this point I am not worried about why haversian bone is weaker than other bone. But when I was taking the specific tensile strength, I made allowance for those haversian systems that were still in the presence of forming by reducing the cross-sectional area of the whole bone by a proportional amount up to the inside of the collagenous bone; but I do not think it will be very significant. If it were significant, this may indicate why haversian bone is weak.

PRITCHARD: It may take 3 months to complete the calcification of a haversian system after all its organic matter has been laid down.

CURREY: Yes. And if one wanted to argue, as people have, that the fact of making haversian bone reduces the strength of the bone part of the time, as it were, this is an argument against this.

Now, against the idea that the location of haversian systems is not related to mechanical needs of the body is the localization not within a bone but between bones.

I took a cat and observed the percentages of haversian bone in various parts of its body (table VI). I compared the long bones with the vertebrae and pelvis, and found that the average amount of remodeling, or haversian bone, in the long bones was about 50 percent and that the average amount in the vertebrae was 87 percent. In other words, much more remodeling had gone on in the compact bone—I am not talking about the spongy areas—of the axial skeleton than in the long bones. This makes good sense mechanically, because if you had a bone of infinite size it would be infinitely strong, but also infinitely

TABLE VI

PERCENTAGES OF HAVERSIAN BONE IN THE CAT SKELETON [a]

Bone	Upper	Middle	Lower
Humerus	40	55	35
Radius	50	55	70 } Mean for fore limb, 57.3
Ulna	80	65	60 }
3d metacarpal		50	
3d digit, 1st phalanx		70	
Femur	40	25	40
Tibia	65	60	60 } Mean for hind limb, 46.4
Fibula	70	0	45 }
3d metatarsal		55	
3d digit, 1st phalanx		50	Mean for limbs, 51.8
1st thoracic vertebra:			
Neural spine		85	
Centrum		85	
Rib		85	
7th thoracic vertebra:			
Neural spine		90	
Centrum		80	
Rib		95	
7th lumbar vertebra: Centrum		90	
1st sacral vertebra		85	
Pelvis:			
Anterior		90	Mean for axial skeleton and
Posterior		80	pelvis, 86.5
Mandible			65 (total)
Petrosal			0 (total)
Parietal			0 (total)
Squamosal			55 (total)

[a] The percentage of the total area occupied by haversian bone in sections from different sites from the skeleton of a mature cat.

heavy. Therefore, you must have a compromise between the weight of the bone and its strength, and you have to allow some kind of safety factor.

When an animal is running along you can make any part heavier or lighter, as you wish. If you make the spine heavier, then you just increase the speed at which it can run by some simple function of its weight. If, however, you make a long bone heavier, you decrease the efficiency of the running by some power of the increase in weight because the legs are moving back and forth; you have to consider the moment of inertia of the distal parts of the long bones, and these are some function of the weight and the square of distance.

I am putting this badly, but what I am getting at is that you can afford the safety factor in the spinal column; for example, when horses fall down and break their legs, they usually do not break their spines. I think this is significant, because the horse is highly adapted to very fast running. The horse clearly has not a great safety factor in its legs.

Therefore, you can say, "If you are going to remodel bone and make a horse run faster, then for heaven's sake do it in the spine, where you have a high safety factor"—that is indeed what one finds.

One could also say, "All right, but remodeling is to provide for the mineral needs of the body. When you use calcium internally, you have to tear some out of the bone and put it back later." This may be so. It does leave a lot of problems. For instance, why remodel within the shaft of the long bone? Why not do it simply at the endosteal border? Because, clearly, if you have a shaft of a long bone which is subjected to bending, the part that needs to be strong is the subperiosteal area around the outside, and the bone becomes of diminishing mechanical importance until you can get into the endosteum. Essentially, the obvious place to get rid of bone is along the inner border. I know, in fact, that there is less remodeling on the outside than the inside. But why is not all remodeling on the inside?

If the purpose of remodeling were the mineral metabolism and if I were Dame Nature, I would put a lump of bone somewhere in the pelvic region, or in some cavity which had no mechanical function whatsoever, and just let that be taken away from or added to as was required.

This does not happen. We use good functional bone, and I think the primary purpose of bone is to enable you to walk around; then you start weakening it by remodeling it.

Another difficulty is that if the purpose of remodeling is to make up the calcium level of the blood, it is strange that there is less remodeling in very small mammals like mice. In these small mammals, the ratio of weight of the skeleton to the weight of the animal as a whole, and presumably of the body fluid, is lower. The bones of a mouse are relatively small, and yet the bones of a mouse do not show haversian systems even though many of them are large enough to do so. Yet these bones have relatively a much larger body of body fluids to support, so that is another difficulty.

Then there is also the strange distribution of haversian systems among the mammals; these systems are found much more in primates and in carnivores than in herbivores. I cannot produce an explanation for this. I cannot see why western Europeans and North Americans, who presumably have a pretty easy time of it from the point of view of their calcium and phosphorus, have a vast amount of remodeling; this remodeling is not simply a function of getting older because bone

starts to be remodeled in humans almost as soon as it is formed. I do not know to what extent we start going into lowered calcium intake; but not very often, I would think.

RAISZ: May I go back for a minute? I think I got lost in something. The idea you gave me before, that haversian remodeling occurred in a bone at a site which was poorly vascularized and had perhaps, therefore, dead cells, would lead me to think that the next thing to test is the tensile strength of the dead bone that you are replacing with the haversian system, rather than the healthy bone around it. That may be impossible to do, but at least you can give us a guess about it.

CURREY: Yes. I think it will probably be lower. I was simply comparing haversian and nonhaversian bone, and did not analyze this. I think this would be possible to do. That is a good point; haversian bone might be making the best of a bad job. Why the bad job is there in the first place is, of course, another matter; I am suggesting that it might be a bad job simply because this is a rather chicken-and-egg thing.

What I have been trying to do is to show that if you look at this from the point of view of the way natural selection works, there are many ways of looking at remodeling. It would seem to me that where the remodeling actually takes place is probably, to some extent at least, determined by where remodeling has already taken place; once remodeling goes on at one place, then, because of the interruption in metabolic processes going on, you are liable to get it going on in that place as well.

MACDONALD: Are there not some stresses other than tensile for which the haversian system is advantageous, just twisting stresses?

CURREY: When you get a torsion fracture, the bone material rather than the femur as a whole has broken in tension. You get a shearing stress that resolves itself into a tension stress at 45° to the direction of the shear. Most bones, of course, are loaded in compression, but the reason that bones actually fail is that they get bent; the part that goes first is the part that is on the convex surface, the part that is under tension.

I think tension is the meaningful stress to consider even though what happens to the bone as a whole may be much more complicated. It may be bending; it may be torsion.

MACDONALD: Was it not the idea that this makes a stronger tube; i.e., the explanation of the importance of the change in direction of the collagen fibers in adjacent lamellae?

CURREY: Yes. That makes it stronger in various directions. You get this in laminar bone. One bit of laminar bone is really like a haversian system split open and spread out. You get this different orientation of collagen fibers.

HEANEY: You indicated that you thought bone had evolved in order to enable us to walk around. Dr. Urist has some thoughts on that.

URIST: The earliest vertebrates had bone only in the exoskeleton. It was dermal bone and used for armor in bottom-dwelling fishes, the ostracoderms which were subject to hydrostatic pressure on all sides. The metabolic activity of exoskeletal bone tissue is probably comparable with that of teeth. After a survey of the microscopic anatomy and the chemical composition of the blood of some representative living lower vertebrates, it occurred to me that the skeleton and the body fluids react as if they were part of a single system. In the course of time and evolution, bone functions as a servo unit, first for an open- and then for a closed-cycle system. Figure 54 (ref. 101) summarizes in diagrammatic form the observations we have made on bone-body continuum.

The earliest forms depended upon the sea for storage of minerals. The reduction in calcium in the blood plasma from 44 to 22 milligrams per 100 ml came at the parting of the ways between marine invertebrates and primitive marine vertebrates. The use for internal control mechanisms and storage of important minerals, such as calcium and phosphorus, decreased total-ion and calcium-ion concentrations of the body fluids. The skeleton became more cellular and capable of active turnover of the body stores of calcium and phosphorus. The first amphibians ventured on land some 300 million years ago when the skeleton contained true bone cells; the body regulatory mechanisms included vitamin D, the parathyroid hormone, as well as the more primitive membrane control, and tissue protein binding of essential ions.

Table VII lists the organs used for homeostasis of calcium ion from the lowest to the highest vertebrates. In the cyclostomes there is no apatite mineral in the skeleton; the supply in the external environment, however, is unlimited. Ionic equilibria are sustained by skin, gill membranes, gut, and kidney by means of an open-cycle system. The serum concentrations of calcium are maintained by mechanisms that act as an ion pump in fresh water and an ion dam in sea water to control unidirectional flow, or movement of ions too rapidly toward the concentration gradient. Hormonal control of calcium metabolism does not appear until the evolution of bone or the storage unit for a closed-cycle or servo system for regulating the turnover of phosphate and calcium ions. Vitamin D appears first in the teleost fishes, and the vitamin D-parathyroid hormone synergism appears first in the amphibians, when the vertebrates came out of the water on to the land.

The elasmobranchs store large quantities of vitamin A in the liver. The teleost fishes that live in a marine habitat do not utilize vitamin D

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MILLIONS OF YEARS	ERA AND PERIOD	CLASS	SKELETON	ION REGULATION	ION CONCENTRATION
58	Cenozoic Eocene	mammalia	bone cells		
165	Mesozoic Jurassic	aves	bone cells		
230	Paleozoic Permian	reptilia	bone cells		
300	Paleozoic Mississippian	amphibia	bone cells	parathyroid hormone and vitamin D	
325	Paleozoic Devonian	teleostei	acellular and cellular bone	vitamin D	300
360	Paleozoic Silurian	marine elasmobranchii	calcified cartilage	membrane phenomena and ground substance	900
425	Paleozoic Ordovician	marine protovertebrates	cartilage		1200
500	Paleozoic Cambrian	marine invertebrates	connective tissue		
600	Precambrian	sea water			

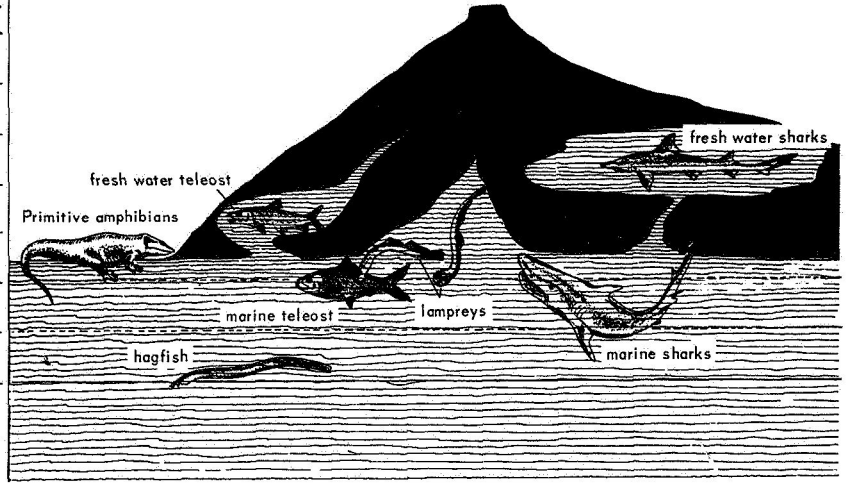

FIGURE 54. Diagram of relationship between the mechanisms of ionic regulation, ion concentrations of the blood, compositions of the skeleton, and differences in the external environment of various classes of vertebrates. [From ref. 101; reprinted by permission of the publisher.]

TABLE VII

Homeostatic Mechanisms for Calcium and Related Ions in the Fluids of the Body

Vertebrate	Integument	Skeletal system	Respiratory system	Endocrine system [a]	Kidney	Liver (vitamin)	Intestinal tract
Mammals and birds	Skin	Bone and cartilage	Lungs	P, T, G, A, PTG	Metanephros	A, D	Gut.
Reptiles	Scales	Bone and cartilage	Lungs	P, T, G, A, PTG	Metanephros	A, D	Gut.
Amphibians	Mucosal skin	Bone and cartilage	Lungs	P, T, G, A, PTG	Mesonephros	A, D	Gut.
Teleosts, fresh water	Scales	Bone and cartilage	Gill membranes	P, T, G, IRB, UBB	Mesonephros	A, D	Gut.
Teleosts, marine	Scales	Bone and cartilage	Gill membranes	P, T, G, IRB	Mesonephros	A	Gut.
Elasmobranchs	Scales	Calcified cartilage.	Gill membranes	P, T, G, IRB	Mesonephros	A	Gut.
Lamprey	Mucosal skin	Cartilage; no mineralized tissues.	Gill membranes	AH, T, G, IRB	Pronephros; many nephrons drain from each segment.	A	Gut.
Hagfish	Mucosal skin	Cartilage; no mineralized tissues.	Gill membranes	AH, T, G, IRB	Pronephros; single pair of nephrons in each segment.	A	Gut.

[a] P—pituitary; T—thyroid; G—gonads; A—adrenals; PTG—parathyroid glands; IRB—interrenal bodies; UBB—ultimobranchial bodies; AH—adenohypophysis.

but store it in the liver. However, when teleosts come into fresh water, their liver stores relatively little vitamin D because it is used for turnover of calcium.

The sturgeon (fig. 55) is an interesting vertebrate in that it is phylogenetically deficient in vitamin D; it has physiologic hypocalcemia. Nearly all of the bone is on the outside of the body in the form of scales, and the calcium in it is probably about as unavailable as the mineral in teeth (fig. 56). Figure 57 is a cross section of this sturgeon showing that the only endoskeletal bone tissue is the bit of tissue at the base of the skull. The blood calcium may be only 7 to 8 mg/100 ml, but the total-ion concentration is the same as any other vertebrate (table VIII). Vertebrate paleontologists say that the sturgeons throughout the course of evolution have lost bone; they contend that fossil sturgeons had lots of bone in the skeleton. The parathyroids presumably came from the ultimobranchial bodies. Apparently the ultimobranchial bodies, the gill arches, and the gills themselves, as Dr. Copp suggested, have something to do with the evolution of both thyroid and parathyroid.

WHEDON: What is the alkaline phosphatase?

URIST: The level of alkaline phosphatase is the same as in any other vertebrate.

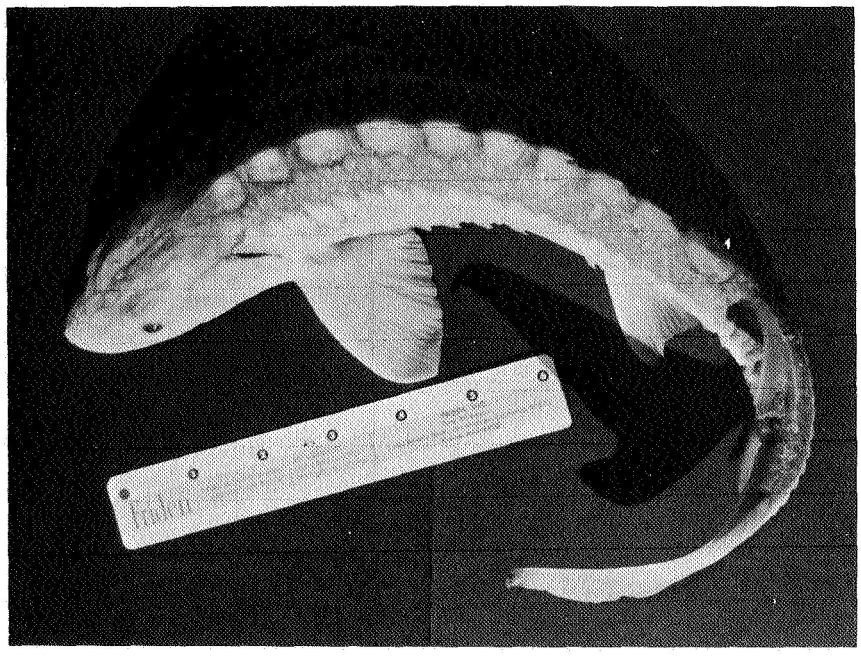

FIGURE 55. Photograph of the young sturgeon, *Acipenser medirosfris* Ayes.

I think that in the story of the evolution of bone and calcium metabolism, the parathyroid hormone plays a role in calcium homeostasis with the development of finer regulation. These hormones appear in vertebrates that exhibit very high-speed swimming, rapid movements, high-energy phosphate metabolism, and terrestrial life. The regulation of the level of calcium, to about 7 or 8 mg/100 ml, in the fluids of the body can go on without vitamin D and without parathyroids. Where thyrocalcitonin appears in the scale of evolution remains to be determined by bioassay of the thyroid of lower forms.

BUDY: How does the haversian system fit into the story?

URIST: Haversian systems may be the mechanism of replacement of aged bone cells by new cells, with or without any relationship to the mechanisms of calcium homeostasis. Once the formation of a resorption cavity gets going, it has no respect for bone; that is, whether the cells are dead or alive. Perhaps the stimulus for resorption comes from an area of one or two dead osteocytes.

The permutations and combinations of factors associated with bone formation and resorption are enormous. Instead of trying to find one

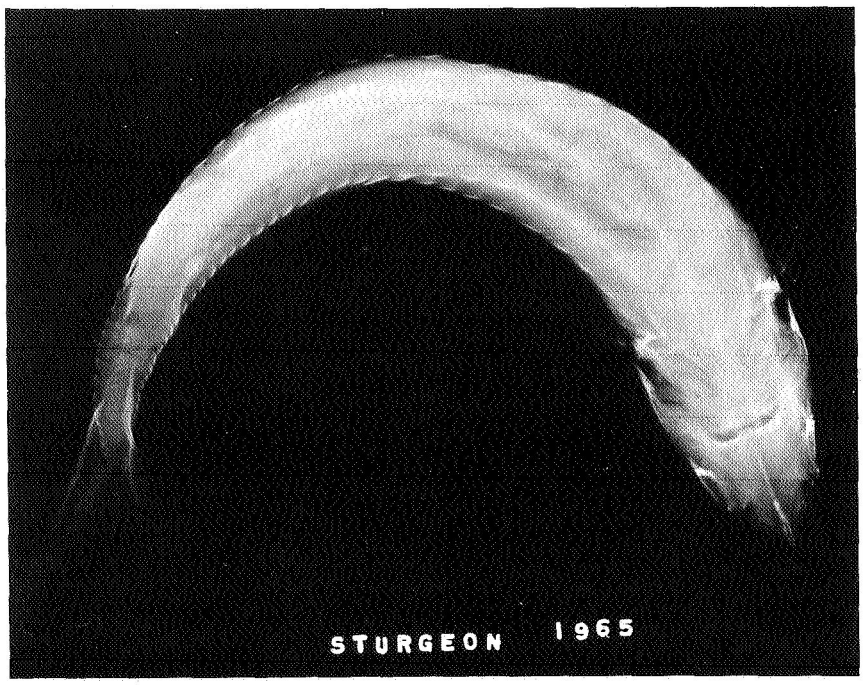

FIGURE 56. Roentgenograph of a sturgeon showing partial armor of rows of plain bony plates. There is very little bone in the endoskeleton, and only in orbital and auditory regions, and the posterior end of the base of the skull. The relationship between the endoskeleton and the exoskeleton is shown in cross section in figure 57.

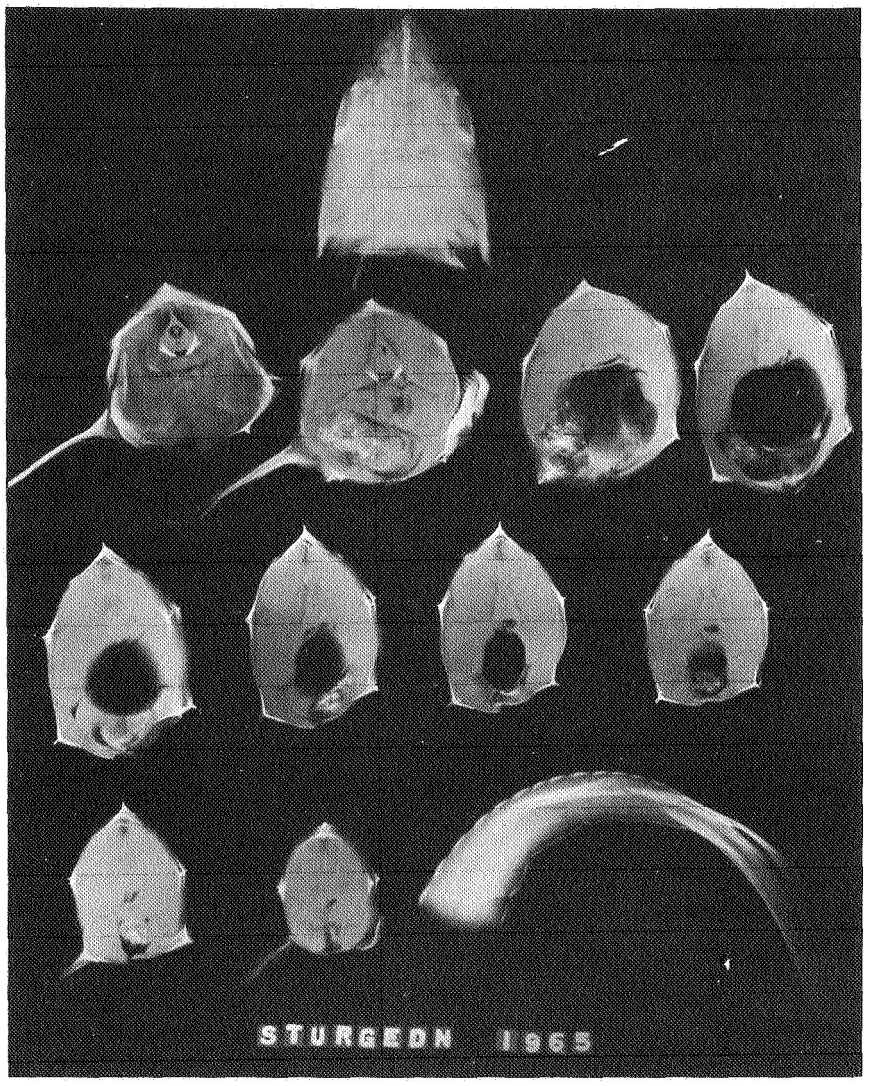

FIGURE 57. Roentgenographs of cross sections of the entire length of the sturgeon shown in figure 56. Note the location of deposits of calcified tissue in the base of the chondrocranium, but none elsewhere in the endoskeleton. The bony plates in the exoskeleton encase the entire body in armor.

explanation, one should look for a combination of factors, both physical and chemical.

HEANEY: Dr. Currey has indicated that he is a zoologist and tends to look at things from the point of view of a naturalist. Would anyone care to hazard an estimate of the selective advantage conferred upon

TABLE VIII

CHEMICAL COMPOSITION OF SERUM OF STURGEON, *Acipenser transmontanus*, IN MARINE AND FRESH WATER HABITAT

Component	Males in marine habitat (San Pablo Bay)	Males and nongravid females in fresh water (Suisun Bay)	Gravid females in fresh water
Sodium.....mmoles/l...	130.0± 15.0	129.0± 11.9	123.0± 10.7
Potassium.....do.....	2.5± 0.4	2.7± 0.5	2.0± 0.3
Calcium.....do.....	1.7± 0.4	1.8± 0.4	4.6± 0.4
Magnesium.....do.....	2.1± 0.2	2.0± 0.4	1.1± 0.1
Chloride.....do.....	115.1± 7.1	111.0± 6.7	116.0± 12.4
Bicarbonate.....do.....	5.2± 0.2	6.0± 0.8	5.0± 0.5
Sulfate.....do.....	0.4± 0.1	0.5± 0.2	0.2± 0.1
Phosphate, inorganic.....do.....	2.9± 0.4	3.3± 0.4	4.1± 0.6
Total.....	259.9± 34.0	256.3± 30.0	256.0± 34.0
Urea nitrogen.....mg/100 ml...	2.8± 0.1	2.5± 0.2	3.0± 0.2
Citric acid.....do.....	0.3± 0.1	0.4± 0.1	0.8± 0.1
Albumin.....g/100 ml...	1.6± 0.1	1.5± 0.1	3.0± 0.4
Globulin.....do.....	1.0± 0.1	1.0± 0.2	1.0± 0.2
Total protein, g/100 ml.....	2.6± 0.1	2.5± 0.2	4.0± 0.4
Alkaline phosphatase, units.....	2.9± 0.3	3.0± 0.4	3.1± 0.4

vertebrates by haversian systems? Obviously, this is a very primitive affair. It looks as if nature is trying to tell us something here, if we could read her handwriting.

BUDY: I remember some microradiographs of dinosaur bone that Jenifer Jowsey had (unpublished data). As I recall, there were many osteons and resorption cavities. Dr. Currey, have you compared dinosaur bone with bone from other species?

CURREY: Dinosaur bone is just like cattle bone. I obtained some dinosaur bone because I was interested in whether, in fact, haversian systems turn the primitive laminar blood supply into a more efficient system, and I plotted the distance of random points from the nearest blood vessel in laminar bone and haversian bone in the same femur. I did exactly the same exercise on fossil dinosaur bone and found the same answer (ref. 102).

BUDY: What about the dugong and the manatee? They also have many osteons and resorption cavities (J. Jowsey, unpublished data).

FREMONT-SMITH: Do they have haversian systems?

URIST: The manatee has haversian systems but, in a relative way, it has a paucity of resorption cavities. Practically all of the bone that is laid down just stays there. The marrow cavities are almost nil. It has what is comparable to osteopetrosis, but not exactly the same thing. It should be called pachyostosis.

HEANEY: Unresorbed calcified cartilage?

NICHOLS: It is pseudohypoparathyroid, really.

URIST: The bones of the adult do not contain large pockets of unresorbed calcified cartilage as in osteopetrosis, but I have not seen the bone of the newborn manatee or dugong.

NICHOLS: I would like to propose an alternative to Drs. McLean and Currey's idea that haversian systems are valuable from the point of view of strength. I think one can postulate another use for them, namely, that haversian systems provide us with a constant supply of new mineral. In support of this just the thinnest thread of data may be cited. We know that as the mineral gets older, the crystals pack a little better, and the strains within them get a little less. We also know from our crystallographer friends that under these circumstances the time and/or the stimulus required to dissolve a given amount of mineral is greater if the mineral is older than if it is younger.

The mouse and the rat, which do not have haversian systems but have pretty good calcium homeostasis, probably use their gut as a potential pool for readily available calcium, as has already been pointed out. Man, however, does not seem to use his gut in this way, but he has very good—indeed probably far better—control. I suspect that man uses his haversian systems as the very rapidly available pool which is needed for such close control.

Dr. McLean says that man has enough other machinery to supply him with the necessary calcium to maintain his serum calcium concentration. This may be true, but I suggest that the newly calcified layer in the newly formed haversian system (in which mineralization is somewhere between 70 and 90 percent of the maximum possible) is the area where there are fresh crystals; where a very small amount of acid poured out will dissolve a lot of mineral in a very short time; and that this area, indeed, is the site where the homeostatic control is really exerted.

HEANEY: That seems all very right, Dr. Nichols, and it makes it very nice that we have haversian systems, but it gives a good bit of credit to the intelligence of the osteoclast. How does the osteoclast know that the organism is getting short on fresh bone and should tunnel out some of this old compact bone so that it can make some more fresh bone?

NICHOLS: May I go on with my theory? I think Dr. Currey has that answer. The osteoclast smells a dead-bone cell.

URIST: If that is true, an osteoclast may function by the same intracellular mechanism as a macrophage or a foreign body giant cell.

HEANEY: I was hoping someone else would say that so I would not have to.

COPP: May I suggest another rather nebulous hypothesis for the distribution of haversian systems? The animals in which you find well-developed haversian systems are carnivores and primates, which normally feed on a diet high in phosphate and low in calcium. This would tend to depress plasma calcium, to stimulate the parathyroids, which in turn would stimulate osteolysis. On the other hand, herbivores normally eat a diet low in phosphate, which would tend to produce hypercalcemia and increased thyrocalcitonin production, with reduced osteolysis.

CURREY: Some such explanation would seem very reasonable. One can check this by looking at primates and carnivores with odd diets. I suppose termites would be high phosphate, would they not?

COPP: High in phosphate no doubt, but rather low in bone.

CURREY: What is a leafy diet?

COPP: A leafy diet is high in calcium and low in phosphate (Ca/P ratio is 2/1 to 5/1) while a meat diet is just the opposite (Ca/P ratio is 1/10 to 1/20).

CURREY: Well now, the colobus monkeys eat leaves almost exclusively, while other monkeys eat nuts and fruits; it would be extremely interesting to compare these two types of monkeys.

PRITCHARD: The herbivores tend to be eating all the time; their stomachs are always full. The carnivores and the higher primates tend to eat three meals or less a day and have long periods of fasting. Homeostasis obviously is more of a problem for these animals than for those that have their belly full all the time.

COPP: Our sheep, for example, are ruminants and absorb nutrients from the rumen almost continuously. It is as if they were fed a continuous intravenous drip, and one must fast them many days to get any sign of starvation.

HEANEY: Dr. Copp, these haversian systems in bone remodeling continue to be present in the absence of the parathyroids, do they not?

COPP: Yes; but osteolysis and remodeling can take place in the absence of the parathyroids.

HEANEY: However, one does not need to interpose the parathyroid, even though it may have an effect.

TALMAGE: I would say one would. The system that the parathyroid might stimulate, which inaugurates the formation of resorption cavities in compact bone, should also work in the absence of the

parathyroid, although at a reduced rate. As an example of this, osteoclast numbers in the metaphysis can be increased by lavaging a rat with a calcium-free rinse. If the animal is parathyroidectomized, this effect is essentially, but not completely, negated. Even in the absence of the parathyroid, stimulus for osteoclast formation remains but is considerably reduced in activity. This should apply also to compact bone where the formation of osteoclasts is the stimulus for the start of a resorption cavity.

NICHOLS: But there is no evidence so far that there is more haversian remodeling in chronic hyperparathyroidism, is there?

TALMAGE: I have no evidence myself, but a year ago I listened to Harold Frost arguing with Lent Johnson for an entire week. It was my impression that both men believed that there was a direct relationship between parathyroid activity and resorption cavities found in compact bone. Dr. McLean does not agree with this, and as I am not an authority I will not push the argument.

COPP: I agree with your point, Dr. Talmage, but it could be that carnivores do have this mechanism; this is the important point. It may be an adaptation to the type of diet.

HEANEY: Dr. Currey has raised a point about osteoclasts being stimulated by, aggravated by, or polarized toward dead or dying cells, and the question of a macrophage function has come up. There are obvious analogies here.

URIST: And the multinucleated giant cell as well.

HEANEY: I wonder if anyone would care to elaborate on this, because it seems to be a crucial point.

URIST: The multinucleated foreign body giant cell resembles the osteoclast both histologically and histochemically so much so that Irving and Handelman (ref. 103) in 1963 were not able to find significant differences between the two. Acid phosphatase, cytochrome oxidase, succinic dehydrogenase, and four other enzyme activities were exactly the same.

HEANEY: Would the cell kinetics people here care to make any comment about the origin of osteoclasts relative to the origin of macrophages?

BÉLANGER: Last summer we ran a short series of experiments, and we obtained a large number of foreign body giant cells in the granuloma pouch, as obtained by Selye's method (ref. 104); when we labeled these animals with radioactive thymidine and counted the number of nuclei that became incorporated within a given time inside these cells as compared with those which formed the osteoclasts, the number of nuclei was the same. The mechanism of formation of these cells appeared to be exactly the same, except that they were away from bone. That was the only difference.

URIST: Dr. Currey's suggestion that dead osteocytes incite bone resorption is supported by our observation on aged women with severe osteoporosis (ref. 105). On the basis of measurements of the thickness of the cortex of the femur, we estimated that aged osteoporotic women had lost 50 percent of their bone mass. Of the remaining 50 percent, half of that mass was dead bone. Dead bone was determined on the basis of cell counts. We wondered whether patients with senile osteoporosis resorb so much bone in an attempt to renew dead bone. The deficit in bone mass is not made up because the capacity to replace lost bone is apparently low in these aged ladies.

BÉLANGER: Also, if we look at sites of bone where foreign elements have become incorporated, these are the sites where a large number of osteoclasts are found, because the bone is abnormal. Wherever there is abnormal bone, which is a result of the cells dying out, or bone malformation as in rickets, or bone that has incorporated unnatural elements, then there are all kinds of osteoclasts.

FREMONT-SMITH: Would you get haversian canals at that time in those areas?

BÉLANGER: I am talking about surfaces in animals that do not have haversian systems.

URIST: Dr. Bélanger, do you regard Neutral Red as a specific stain for osteoclasts?

BÉLANGER: I do not know what part of the osteoclast you want to stain, but certainly it is easy to distinguish an osteoclast from a dead piece of bone if we use, for example, the Wright stain combination that I mentioned this morning. Bone will pick up the acid dye, eosin, whereas the osteoclast will always pick up the blue or azure component; it is very easy to see the osteoblast as well. Some people (including myself) have expressed the opinion that osteoclasts were just chunks of detached dead bone, but it is very easily shown that they are not, just by that simple method of staining.

BUDY: Dr. Bélanger mentioned the relationship of abnormal bone and osteoclasts. I think giant cells can also be included.

When one administers parathyroid extract to an incisor-absent mutant rat, as shown in figure 58, a marked change in the cell population occurs (fig. 59). There is a rapid mobilization of cells; osteoclasts are considerably larger than those observed in the untreated incisor-absent rat. They have many nuclei, and often one sees large vacuoles in the cytoplasm. The change is quite rapid, as though the stimulus for bone resorption caused relatively quiescent cells to become active resorbers.

In addition to the osteoclasts there is another type of cell, which I will call a giant cell for want of a better name. These cells have from 10 to 15 nuclei, are basophilic, but are not always adjacent to bone

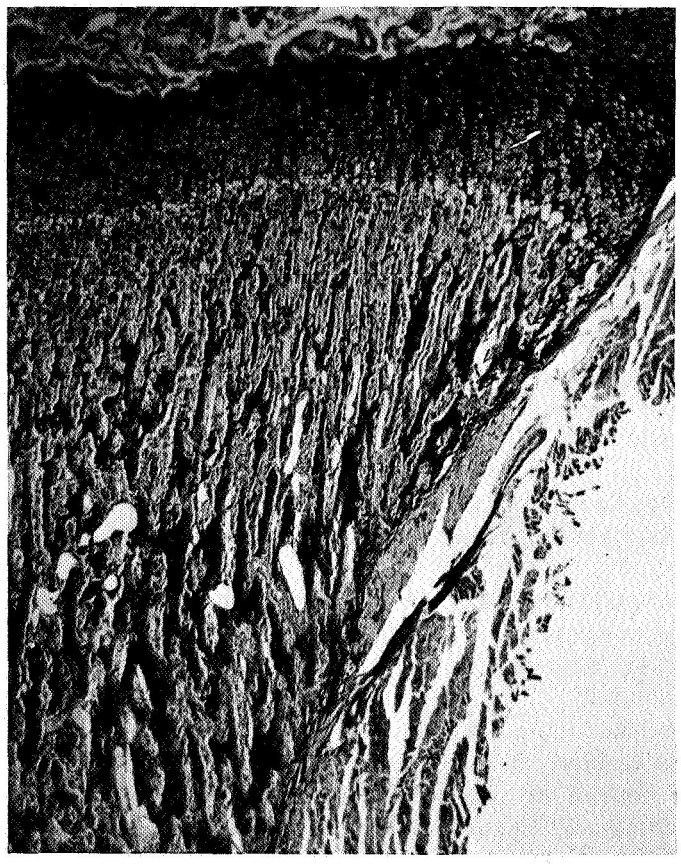

FIGURE 58. Photomicrograph of a section of the tibia from an *ia* (incisor-absent) rat, 5 weeks old. The elongated spongiosa is one of the characteristics of this mutant strain of rats. Osteoclasts are present, but are somewhat inactive since resorption does go on, but at a reduced rate. Formalin fixation; HEA stain. 25 ×.

spicules. They are present along the endosteum and can also be seen in the metaphysis where widespread resorption of trabeculae has taken place.

These are not macrophages; their location and appearance exclude the possibility of their being megakaryocytes, which are numerous in these rats. The giant cells do not have a polymorphous nucleus characteristic of megakaryocytes, but contain many distinct nuclei. With HEA stain these cells are basophilic and are not granular; but they do have many vacuoles in the cytoplasm.

Ordinarily, there are many variations in osteoclasts in these mutant rats; however, one does not see so many nuclei until the animal is

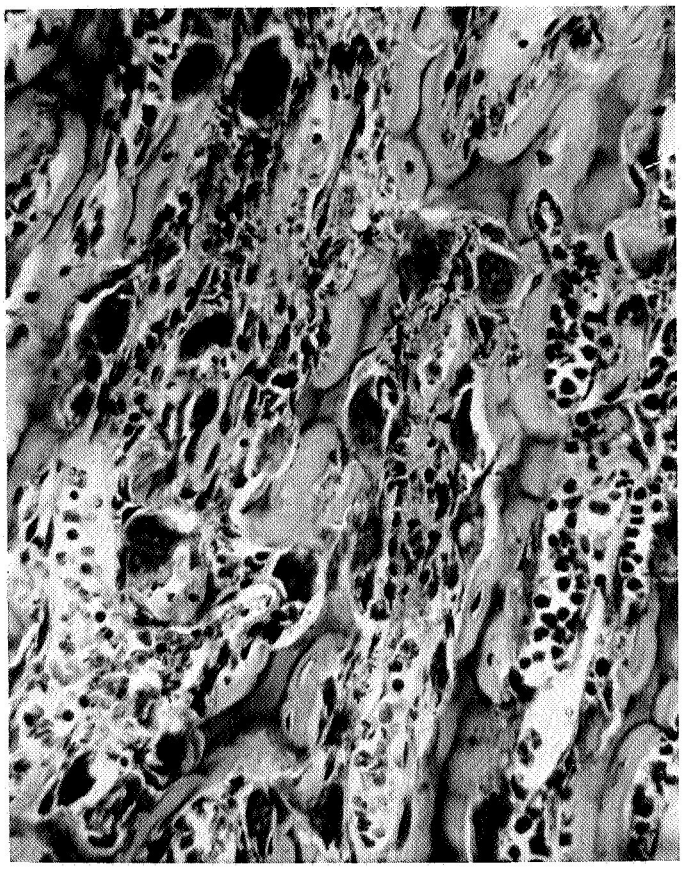

FIGURE 59. Photomicrograph of a section of the tibia from an *ia* rat following administration of 400 units of parathyroid extract (100 units daily for 2 days, then 50 units for 4 days). The littermate of this rat is shown in figure 58. Note the osteoclasts surrounding spicules of bone. After the spicules have been resorbed, these cells remain in the marrow and along the endosteum. Formalin fixation; HEA stain. 250 ×.

stressed with PTE, for example. The picture is very different from that seen in a normal, nonmutant intact rat after PTE administration. (See figs. 108 to 112.) If these giant cells are osteoclasts, then they have assumed a new form to enable them to control their environment efficiently under stress. Once the initial stress is over, they continue in the same form and remain in fibrotic areas or in the marrow where bone spicules might have been present.

BELANGER: What is the treatment here?

BUDY: Lilly's parathyroid extract, 400 units over 6 days.

NICHOLS: Dr. Budy, did they get hypercalcemic?

BUDY: We did not collect blood on this group. I do not know.

ARNAUD: If you give them enough purified parathyroid hormone, both a calcemic and a phosphaturic effect can be observed.

URIST: There are at least two schools of thought on the origin of osteoclasts; one is the idea of Jee and Nolan (ref. 106) that the osteoclast can come from a fusion of macrophages, and the other is the idea of Fischman and Hay (ref. 107) in which the origin of nuclei of an osteoclast of a salamander is traced as far back as the monocytes of blood. Apparently, the question of cell origin depends on how far back into the pool of the cells of primitive mesenchyme one wants to go. One can suppose that every specialized connective tissue cell in the body can come from either a monocyte or a mesenchymal cell.

BÉLANGER: They come from primitive cells, osteoprogenitors, as Dr. Young calls them.

URIST: Yes; the progeny of a monocyte may enter the cycle of bone cells and become an "osteoclastoprogenitor" cell.

HEANEY: We have said nothing about blood supply except as it relates to cell nutrition. Dr. Whedon, do you have any thoughts on this?

WHEDON: No. I have a question. I have been confused through the years as to the effects of venous ligation, sympathectomy, and interference with arterial supply on bone. In some cases there seems to be increased formation and in other cases, increased bone resorption; I wondered if anyone here had some modern and reliable information on the effect of circulation on bone reconstruction.

HOLTZER: I would like to repropose the experiment that I mentioned earlier. Why could you not take something like a mouse or a chick and graft a lot of bone to its muscle, then let 1 or 2 years go by? I am sure you would have a lot of ectopic pieces of bone sitting around. Then look at the haversian system——

HEANEY: Is this the organ you are speaking about, this nonstructural calcium mass embedded in tissue which would be available for homeostatic purposes?

URIST: The crayfish has that—the gastrolith. The crayfish makes a big lump of calcium and transports that calcium into the shell when he is ready for it. So, vertebrates can do it.

HOLTZER: That does not answer my question. Has anyone taken a piece of bone or several of them and grafted them into four or five hunks of muscle to actually trace the haversian system with time?

HEANEY: The haversian systems where?

HOLTZER: In the grafted bone. You posed a problem. I am trying to set up an experiment. If somebody has done it, I would be delighted to know about it. You take a dozen pieces of bone and put

them in a dozen different muscular sites. Then you check those, perhaps 6 months later. What are the haversian system patterns?

URIST: The bone would die.

HEANEY: The bone graft dies initially, but it is invaded then by host cells which ultimately absorb it.

URIST: We are going to discuss that at the next session.